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[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF BREAST CANCER

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1      HFFPQLGQGVYRPLYPFRFVAAGCGGUSACGERSGFGAAGSELAAGGSI  R1002Cof.F.TWO
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3
4      LFFVLSGVYUAPFAAAAAAAGGTCATLPTATELCTFTQLGAGUELELD  R1002Cof.F.TWO
5      Iroquois.FRO
6
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12
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17     Iroquois.FRO
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101    Iroquois.FRO

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(57) Abstract: Compositions and methods for the therapy and diagnosis of cancer, such as breast cancer, are disclosed. Compositions may comprise one or more breast tumor proteins, immunogenic portions thereof, or polynucleotides that encode such portions. Alternatively, a therapeutic composition may comprise an antigen presenting cell that expresses a breast tumor protein, or a T cell that is specific for cells expressing such a protein. Such compositions may be used, for example, for the prevention and treatment of diseases such as breast cancer. Diagnostic methods based on detecting a breast tumor protein, or mRNA encoding such a protein, in a sample are also provided.

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COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF BREAST CANCER

TECHNICAL FIELD OF THE INVENTION

The present invention relates generally to therapy and diagnosis of
5 cancer, such as breast cancer. The invention is more specifically related to polypeptides
comprising at least a portion of a breast tumor protein, and to polynucleotides encoding
such polypeptides. Such polypeptides and polynucleotides may be used in vaccines and
pharmaceutical compositions for prevention and treatment of breast cancer, and for the
diagnosis and monitoring of such cancers.

10 BACKGROUND OF THE INVENTION

Breast cancer is a significant health problem for women in the United
States and throughout the world. Although advances have been made in detection and
treatment of the disease, breast cancer remains the second leading cause of cancer-
related deaths in women, affecting more than 180,000 women in the United States each
15 year. For women in North America, the life-time odds of getting breast cancer are now
one in eight.

No vaccine or other universally successful method for the prevention or
treatment of breast cancer is currently available. Management of the disease currently
relies on a combination of early diagnosis (through routine breast screening procedures)
20 and aggressive treatment, which may include one or more of a variety of treatments
such as surgery, radiotherapy, chemotherapy and hormone therapy. The course of
treatment for a particular breast cancer is often selected based on a variety of prognostic
parameters, including an analysis of specific tumor markers. *See, e.g.,* Porter-Jordan
and Lippman, *Breast Cancer* 8:73-100, 1994. However, the use of established markers
25 often leads to a result that is difficult to interpret, and the high mortality observed in
breast cancer patients indicates that improvements are needed in the treatment,
diagnosis and prevention of the disease.

Immunotherapies have the potential to substantially improve breast
cancer treatment and survival. Such therapies may involve the generation or

enhancement of an immune response to a breast tumor antigen. However, to date, relatively few breast tumor antigens are known and the generation of an immune response against such antigens has not been shown to be therapeutically beneficial.

Accordingly, there is a need in the art for improved methods for
5 identifying breast tumor antigens and for using such antigens in the diagnosis and therapy of breast cancer. The present invention fulfills these needs and further provides other related advantages.

SUMMARY OF THE INVENTION

Briefly stated, the present invention provides compositions and methods
10 for the diagnosis and therapy of cancer, such as breast cancer. In one aspect, the present invention provides polypeptides comprising at least a portion of a breast tumor protein, or a variant thereof. Certain portions and other variants are immunogenic, such that the ability of the variant to react with antigen-specific antisera is not substantially diminished. Within certain embodiments, the polypeptide comprises a sequence that is
15 encoded by a polynucleotide sequence selected from the group consisting of sequences recited in SEQ ID NOs:1-125, variants of such sequences and complements of such sequences. One such polypeptide comprises a sequence recited in SEQ ID NO:126, or a variant thereof that is at least 90% identical to SEQ ID NO:126.

The present invention further provides polynucleotides that encode a
20 polypeptide as described above, or a portion thereof (such as a portion encoding at least 9, preferably at least 15, amino acid residues of a breast tumor protein), expression vectors comprising such polynucleotides and host cells transformed or transfected with such expression vectors.

Within other aspects, the present invention provides pharmaceutical
25 compositions comprising a polypeptide or polynucleotide as described above and a physiologically acceptable carrier.

Within a related aspect of the present invention, vaccines are provided. Such vaccines comprise a polypeptide or polynucleotide as described above and a non-specific immune response enhancer.

The present invention further provides pharmaceutical compositions that comprise: (a) an antibody or antigen-binding fragment thereof that specifically binds to a breast tumor protein; and (b) a physiologically acceptable carrier.

5 Within further aspects, the present invention provides pharmaceutical compositions comprising: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a pharmaceutically acceptable carrier or excipient. Antigen presenting cells include dendritic cells, macrophages and B cells.

10 Within related aspects, vaccines are provided that comprise: (a) an antigen presenting cell that expresses a polypeptide as described above and (b) a non-specific immune response enhancer.

The present invention further provides, in other aspects, fusion proteins that comprise at least one polypeptide as described above, as well as polynucleotides encoding such fusion proteins.

15 Within related aspects, pharmaceutical compositions comprising a fusion protein, or a polynucleotide encoding a fusion protein, in combination with a physiologically acceptable carrier are provided.

Vaccines are further provided, within other aspects, that comprise a fusion protein or a polynucleotide encoding a fusion protein in combination with a non-specific immune response enhancer.

20 Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient a pharmaceutical composition or vaccine as recited above.

25 The present invention further provides, within other aspects, methods for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a breast tumor protein, wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the protein from the sample.

30 Within related aspects, methods are provided for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated as described above.

Methods are further provided, within other aspects, for stimulating and/or expanding T cells specific for a breast tumor protein, comprising contacting T cells with one or more of: (i) a polypeptide as described above; (ii) a polynucleotide encoding such a polypeptide; and/or (iii) an antigen presenting cell that expresses such a polypeptide; under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells. Isolated T cell populations comprising T cells prepared as described above are also provided.

Within further aspects, the present invention provides methods for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population as described above.

The present invention further provides methods for inhibiting the development of a cancer in a patient, comprising the steps of: (a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with one or more of: (i) a polypeptide comprising at least an immunogenic portion of a breast tumor protein; (ii) a polynucleotide encoding such a polypeptide; and (iii) an antigen-presenting cell that expresses such a polypeptide; and (b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient. Proliferated cells may, but need not, be cloned prior to administration to the patient.

Within further aspects, the present invention provides methods for determining the presence or absence of a cancer in a patient, comprising (a) contacting a biological sample obtained from a patient with a binding agent that binds to a polypeptide as recited above; (b) detecting in the sample an amount of polypeptide that binds to the binding agent; and (c) comparing the amount of polypeptide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within preferred embodiments, the binding agent is an antibody, more preferably a monoclonal antibody. The cancer may be breast cancer.

The present invention also provides, within other aspects, methods for monitoring the progression of a cancer in a patient. Such methods comprise the steps of: (a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a polypeptide as recited above; (b) detecting in the

sample an amount of polypeptide that binds to the binding agent; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polypeptide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

The present invention further provides, within other aspects, methods for determining the presence or absence of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein; (b) detecting in the sample a level of a polynucleotide, preferably mRNA, that hybridizes to the oligonucleotide; and (c) comparing the level of polynucleotide that hybridizes to the oligonucleotide with a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient. Within certain embodiments, the amount of mRNA is detected via polymerase chain reaction using, for example, at least one oligonucleotide primer that hybridizes to a polynucleotide encoding a polypeptide as recited above, or a complement of such a polynucleotide. Within other embodiments, the amount of mRNA is detected using a hybridization technique, employing an oligonucleotide probe that hybridizes to a polynucleotide that encodes a polypeptide as recited above, or a complement of such a polynucleotide.

In related aspects, methods are provided for monitoring the progression of a cancer in a patient, comprising the steps of: (a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein; (b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; (c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and (d) comparing the amount of polynucleotide detected in step (c) with the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

Within further aspects, the present invention provides antibodies, such as monoclonal antibodies, that bind to a polypeptide as described above, as well as

diagnostic kits comprising such antibodies. Diagnostic kits comprising one or more oligonucleotide probes or primers as described above are also provided.

These and other aspects of the present invention will become apparent upon reference to the following detailed description and attached drawings. All
5 references disclosed herein are hereby incorporated by reference in their entirety as if each was incorporated individually.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 represents a sequence alignment between the representative breast tumor polypeptide B1002C (SEQ ID NO:126) and the mouse iroquois homeobox
10 protein 3.

DETAILED DESCRIPTION OF THE INVENTION

As noted above, the present invention is generally directed to compositions and methods for using the compositions, for example in the therapy and diagnosis of cancer, such as breast cancer. Certain illustrative compositions described
15 herein include breast tumor polypeptides, polynucleotides encoding such polypeptides, binding agents such as antibodies, antigen presenting cells (APCs) and/or immune system cells (e.g., T cells). A "breast tumor protein," as the term is used herein, refers generally to a protein that is expressed in breast tumor cells at a level that is at least two fold, and preferably at least five fold, greater than the level of expression in a normal
20 tissue, as determined using a representative assay provided herein. Certain breast tumor proteins are tumor proteins that react detectably (within an immunoassay, such as an ELISA or Western blot) with antisera of a patient afflicted with breast cancer.

Therefore, in accordance with the above, and as described further below, the present invention provides illustrative polynucleotide compositions having
25 sequences set forth in SEQ ID NO:1-125, illustrative polypeptide compositions having amino acid sequences set forth in SEQ ID NO:126, antibody compositions capable of binding such polypeptides, and numerous additional embodiments employing such compositions, for example in the detection, diagnosis and/or therapy of human breast cancer.

POLYNUCLEOTIDE COMPOSITIONS

As used herein, the terms "DNA segment" and "polynucleotide" refer to a DNA molecule that has been isolated free of total genomic DNA of a particular species. Therefore, a DNA segment encoding a polypeptide refers to a DNA segment that contains one or more coding sequences yet is substantially isolated away from, or purified free from, total genomic DNA of the species from which the DNA segment is obtained. Included within the terms "DNA segment" and "polynucleotide" are DNA segments and smaller fragments of such segments, and also recombinant vectors, including, for example, plasmids, cosmids, phagemids, phage, viruses, and the like.

As will be understood by those skilled in the art, the DNA segments of this invention can include genomic sequences, extra-genomic and plasmid-encoded sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, peptides and the like. Such segments may be naturally isolated, or modified synthetically by the hand of man.

"Isolated," as used herein, means that a polynucleotide is substantially away from other coding sequences, and that the DNA segment does not contain large portions of unrelated coding DNA, such as large chromosomal fragments or other functional genes or polypeptide coding regions. Of course, this refers to the DNA segment as originally isolated, and does not exclude genes or coding regions later added to the segment by the hand of man.

As will be recognized by the skilled artisan, polynucleotides may be single-stranded (coding or antisense) or double-stranded, and may be DNA (genomic, cDNA or synthetic) or RNA molecules. RNA molecules include HnRNA molecules, which contain introns and correspond to a DNA molecule in a one-to-one manner, and mRNA molecules, which do not contain introns. Additional coding or non-coding sequences may, but need not, be present within a polynucleotide of the present invention, and a polynucleotide may, but need not, be linked to other molecules and/or support materials.

Polynucleotides may comprise a native sequence (*i.e.*, an endogenous sequence that encodes a breast tumor protein or a portion thereof) or may comprise a variant, or a biological or antigenic functional equivalent of such a sequence.

Polynucleotide variants may contain one or more substitutions, additions, deletions and/or insertions, as further described below, preferably such that the immunogenicity of the encoded polypeptide is not diminished, relative to a native tumor protein. The effect on the immunogenicity of the encoded polypeptide may generally be assessed as described herein. The term "variants" also encompasses homologous genes of xenogenic origin.

When comparing polynucleotide or polypeptide sequences, two sequences are said to be "identical" if the sequence of nucleotides or amino acids in the two sequences is the same when aligned for maximum correspondence, as described below. Comparisons between two sequences are typically performed by comparing the sequences over a comparison window to identify and compare local regions of sequence similarity. A "comparison window" as used herein, refers to a segment of at least about 20 contiguous positions, usually 30 to about 75, 40 to about 50, in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned.

Optimal alignment of sequences for comparison may be conducted using the Megalign program in the Lasergene suite of bioinformatics software (DNASTAR, Inc., Madison, WI), using default parameters. This program embodies several alignment schemes described in the following references: Dayhoff, M.O. (1978) A model of evolutionary change in proteins – Matrices for detecting distant relationships. In Dayhoff, M.O. (ed.) Atlas of Protein Sequence and Structure, National Biomedical Research Foundation, Washington DC Vol. 5, Suppl. 3, pp. 345-358; Hein J. (1990) Unified Approach to Alignment and Phylogenies pp. 626-645 *Methods in Enzymology* vol. 183, Academic Press, Inc., San Diego, CA; Higgins, D.G. and Sharp, P.M. (1989) *CABIOS* 5:151-153; Myers, E.W. and Muller W. (1988) *CABIOS* 4:11-17; Robinson, E.D. (1971) *Comb. Theor* 11:105; Santou, N. Nes, M. (1987) *Mol. Biol. Evol.* 4:406-425; Sneath, P.H.A. and Sokal, R.R. (1973) *Numerical Taxonomy – the Principles and Practice of Numerical Taxonomy*, Freeman Press, San Francisco, CA; Wilbur, W.J. and Lipman, D.J. (1983) *Proc. Natl. Acad., Sci. USA* 80:726-730.

Alternatively, optimal alignment of sequences for comparison may be conducted by the local identity algorithm of Smith and Waterman (1981) *Add. APL*.

Math 2:482, by the identity alignment algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443, by the search for similarity methods of Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. USA* 85: 2444, by computerized implementations of these algorithms (GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics
5 Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, WI), or by inspection.

One preferred example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1977) *Nucl. Acids Res.* 25:3389-3402
10 and Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-410, respectively. BLAST and BLAST 2.0 can be used, for example with the parameters described herein, to determine percent sequence identity for the polynucleotides and polypeptides of the invention. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. In one illustrative example, cumulative scores can be
15 calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix can be used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved
20 value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, and expectation (E) of 10, and the BLOSUM62 scoring matrix
25 (see Henikoff and Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915) alignments, (B) of 50, expectation (E) of 10, M=5, N=-4 and a comparison of both strands.

Preferably, the "percentage of sequence identity" is determined by comparing two optimally aligned sequences over a window of comparison of at least 20 positions, wherein the portion of the polynucleotide or polypeptide sequence in the
30 comparison window may comprise additions or deletions (*i.e.*, gaps) of 20 percent or less, usually 5 to 15 percent, or 10 to 12 percent, as compared to the reference

sequences (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid bases or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the reference sequence (*i.e.*, the window size) and multiplying the results by 100 to yield the percentage of sequence identity.

Therefore, the present invention encompasses polynucleotide and polypeptide sequences having substantial identity to the sequences disclosed herein, for example those comprising at least 50% sequence identity, preferably at least 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% or higher, sequence identity compared to a polynucleotide or polypeptide sequence of this invention using the methods described herein, (*e.g.*, BLAST analysis using standard parameters, as described below). One skilled in this art will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like.

In additional embodiments, the present invention provides isolated polynucleotides and polypeptides comprising various lengths of contiguous stretches of sequence identical to or complementary to one or more of the sequences disclosed herein. For example, polynucleotides are provided by this invention that comprise at least about 15, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500 or 1000 or more contiguous nucleotides of one or more of the sequences disclosed herein as well as all intermediate lengths there between. It will be readily understood that "intermediate lengths", in this context, means any length between the quoted values, such as 16, 17, 18, 19, *etc.*; 21, 22, 23, *etc.*; 30, 31, 32, *etc.*; 50, 51, 52, 53, *etc.*; 100, 101, 102, 103, *etc.*; 150, 151, 152, 153, *etc.*; including all integers through 200-500; 500-1,000, and the like.

The polynucleotides of the present invention, or fragments thereof, regardless of the length of the coding sequence itself, may be combined with other DNA sequences, such as promoters, polyadenylation signals, additional restriction enzyme sites, multiple cloning sites, other coding segments, and the like, such that their

overall length may vary considerably. It is therefore contemplated that a nucleic acid fragment of almost any length may be employed, with the total length preferably being limited by the ease of preparation and use in the intended recombinant DNA protocol. For example, illustrative DNA segments with total lengths of about 10,000, about 5000, about 3000, about 2,000, about 1,000, about 500, about 200, about 100, about 50 base pairs in length, and the like, (including all intermediate lengths) are contemplated to be useful in many implementations of this invention.

In other embodiments, the present invention is directed to polynucleotides that are capable of hybridizing under moderately stringent conditions to a polynucleotide sequence provided herein, or a fragment thereof, or a complementary sequence thereof. Hybridization techniques are well known in the art of molecular biology. For purposes of illustration, suitable moderately stringent conditions for testing the hybridization of a polynucleotide of this invention with other polynucleotides include prewashing in a solution of 5 X SSC, 0.5% SDS, 1.0 mM EDTA (pH 8.0); hybridizing at 50°C-65°C, 5 X SSC, overnight; followed by washing twice at 65°C for 20 minutes with each of 2X, 0.5X and 0.2X SSC containing 0.1% SDS.

Moreover, it will be appreciated by those of ordinary skill in the art that, as a result of the degeneracy of the genetic code, there are many nucleotide sequences that encode a polypeptide as described herein. Some of these polynucleotides bear minimal homology to the nucleotide sequence of any native gene. Nonetheless, polynucleotides that vary due to differences in codon usage are specifically contemplated by the present invention. Further, alleles of the genes comprising the polynucleotide sequences provided herein are within the scope of the present invention. Alleles are endogenous genes that are altered as a result of one or more mutations, such as deletions, additions and/or substitutions of nucleotides. The resulting mRNA and protein may, but need not, have an altered structure or function. Alleles may be identified using standard techniques (such as hybridization, amplification and/or database sequence comparison).

PROBES AND PRIMERS

In other embodiments of the present invention, the polynucleotide sequences provided herein can be advantageously used as probes or primers for nucleic acid hybridization. As such, it is contemplated that nucleic acid segments that comprise
5 a sequence region of at least about 15 nucleotide long contiguous sequence that has the same sequence as, or is complementary to, a 15 nucleotide long contiguous sequence disclosed herein will find particular utility. Longer contiguous identical or complementary sequences, *e.g.*, those of about 20, 30, 40, 50, 100, 200, 500, 1000 (including all intermediate lengths) and even up to full length sequences will also be of
10 use in certain embodiments.

The ability of such nucleic acid probes to specifically hybridize to a sequence of interest will enable them to be of use in detecting the presence of complementary sequences in a given sample. However, other uses are also envisioned, such as the use of the sequence information for the preparation of mutant species
15 primers, or primers for use in preparing other genetic constructions.

Polynucleotide molecules having sequence regions consisting of contiguous nucleotide stretches of 10-14, 15-20, 30, 50, or even of 100-200 nucleotides or so (including intermediate lengths as well), identical or complementary to a polynucleotide sequence disclosed herein, are particularly contemplated as
20 hybridization probes for use in, *e.g.*, Southern and Northern blotting. This would allow a gene product, or fragment thereof, to be analyzed, both in diverse cell types and also in various bacterial cells. The total size of fragment, as well as the size of the complementary stretch(es), will ultimately depend on the intended use or application of the particular nucleic acid segment. Smaller fragments will generally find use in
25 hybridization embodiments, wherein the length of the contiguous complementary region may be varied, such as between about 15 and about 100 nucleotides, but larger contiguous complementarity stretches may be used, according to the length complementary sequences one wishes to detect.

The use of a hybridization probe of about 15-25 nucleotides in length
30 allows the formation of a duplex molecule that is both stable and selective. Molecules having contiguous complementary sequences over stretches greater than 15 bases in

length are generally preferred, though, in order to increase stability and selectivity of the hybrid, and thereby improve the quality and degree of specific hybrid molecules obtained. One will generally prefer to design nucleic acid molecules having gene-complementary stretches of 15 to 25 contiguous nucleotides, or even longer where
5 desired.

Hybridization probes may be selected from any portion of any of the sequences disclosed herein. All that is required is to review the sequence set forth in SEQ ID NO:1-125, or to any continuous portion of the sequence, from about 15-25 nucleotides in length up to and including the full length sequence, that one wishes to
10 utilize as a probe or primer. The choice of probe and primer sequences may be governed by various factors. For example, one may wish to employ primers from towards the termini of the total sequence.

Small polynucleotide segments or fragments may be readily prepared by, for example, directly synthesizing the fragment by chemical means, as is commonly
15 practiced using an automated oligonucleotide synthesizer. Also, fragments may be obtained by application of nucleic acid reproduction technology, such as the PCR™ technology of U. S. Patent 4,683,202 (incorporated herein by reference), by introducing selected sequences into recombinant vectors for recombinant production, and by other recombinant DNA techniques generally known to those of skill in the art of molecular
20 biology.

The nucleotide sequences of the invention may be used for their ability to selectively form duplex molecules with complementary stretches of the entire gene or gene fragments of interest. Depending on the application envisioned, one will typically desire to employ varying conditions of hybridization to achieve varying degrees of
25 selectivity of probe towards target sequence. For applications requiring high selectivity, one will typically desire to employ relatively stringent conditions to form the hybrids, *e.g.*, one will select relatively low salt and/or high temperature conditions, such as provided by a salt concentration of from about 0.02 M to about 0.15 M salt at temperatures of from about 50°C to about 70°C. Such selective conditions tolerate
30 little, if any, mismatch between the probe and the template or target strand, and would be particularly suitable for isolating related sequences.

Of course, for some applications, for example, where one desires to prepare mutants employing a mutant primer strand hybridized to an underlying template, less stringent (reduced stringency) hybridization conditions will typically be needed in order to allow formation of the heteroduplex. In these circumstances, one
5 may desire to employ salt conditions such as those of from about 0.15 M to about 0.9 M salt, at temperatures ranging from about 20°C to about 55°C. Cross-hybridizing species can thereby be readily identified as positively hybridizing signals with respect to control hybridizations. In any case, it is generally appreciated that conditions can be rendered
10 more stringent by the addition of increasing amounts of formamide, which serves to destabilize the hybrid duplex in the same manner as increased temperature. Thus, hybridization conditions can be readily manipulated, and thus will generally be a method of choice depending on the desired results.

POLYNUCLEOTIDE IDENTIFICATION AND CHARACTERIZATION

Polynucleotides may be identified, prepared and/or manipulated using
15 any of a variety of well established techniques. For example, a polynucleotide may be identified, as described in more detail below, by screening a microarray of cDNAs for tumor-associated expression (*i.e.*, expression that is at least two fold greater in a tumor than in normal tissue, as determined using a representative assay provided herein). Such screens may be performed, for example, using a Synteni microarray (Palo Alto,
20 CA) according to the manufacturer's instructions (and essentially as described by Schena *et al.*, *Proc. Natl. Acad. Sci. USA* 93:10614-10619, 1996 and Heller *et al.*, *Proc. Natl. Acad. Sci. USA* 94:2150-2155, 1997). Alternatively, polynucleotides may be amplified from cDNA prepared from cells expressing the proteins described herein, such as breast tumor cells. Such polynucleotides may be amplified via polymerase
25 chain reaction (PCR). For this approach, sequence-specific primers may be designed based on the sequences provided herein, and may be purchased or synthesized.

An amplified portion of a polynucleotide of the present invention may be used to isolate a full length gene from a suitable library (*e.g.*, a breast tumor cDNA library) using well known techniques. Within such techniques, a library (cDNA or
30 genomic) is screened using one or more polynucleotide probes or primers suitable for

amplification. Preferably, a library is size-selected to include larger molecules. Random primed libraries may also be preferred for identifying 5' and upstream regions of genes. Genomic libraries are preferred for obtaining introns and extending 5' sequences.

5 For hybridization techniques, a partial sequence may be labeled (*e.g.*, by nick-translation or end-labeling with ^{32}P) using well known techniques. A bacterial or bacteriophage library is then generally screened by hybridizing filters containing denatured bacterial colonies (or lawns containing phage plaques) with the labeled probe (*see* Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor
10 Laboratories, Cold Spring Harbor, NY, 1989). Hybridizing colonies or plaques are selected and expanded, and the DNA is isolated for further analysis. cDNA clones may be analyzed to determine the amount of additional sequence by, for example, PCR using a primer from the partial sequence and a primer from the vector. Restriction maps and partial sequences may be generated to identify one or more overlapping
15 clones. The complete sequence may then be determined using standard techniques, which may involve generating a series of deletion clones. The resulting overlapping sequences can then be assembled into a single contiguous sequence. A full length cDNA molecule can be generated by ligating suitable fragments, using well known techniques.

Alternatively, there are numerous amplification techniques for obtaining
20 a full length coding sequence from a partial cDNA sequence. Within such techniques, amplification is generally performed via PCR. Any of a variety of commercially available kits may be used to perform the amplification step. Primers may be designed using, for example, software well known in the art. Primers are preferably 22-30 nucleotides in length, have a GC content of at least 50% and anneal to the target
25 sequence at temperatures of about 68°C to 72°C. The amplified region may be sequenced as described above, and overlapping sequences assembled into a contiguous sequence.

One such amplification technique is inverse PCR (*see* Triglia *et al.*, *Nucl. Acids Res.* 16:8186, 1988), which uses restriction enzymes to generate a fragment
30 in the known region of the gene. The fragment is then circularized by intramolecular ligation and used as a template for PCR with divergent primers derived from the known

region. Within an alternative approach, sequences adjacent to a partial sequence may be retrieved by amplification with a primer to a linker sequence and a primer specific to a known region. The amplified sequences are typically subjected to a second round of amplification with the same linker primer and a second primer specific to the known region. A variation on this procedure, which employs two primers that initiate extension in opposite directions from the known sequence, is described in WO 96/38591. Another such technique is known as "rapid amplification of cDNA ends" or RACE. This technique involves the use of an internal primer and an external primer, which hybridizes to a polyA region or vector sequence, to identify sequences that are 5' and 3' of a known sequence. Additional techniques include capture PCR (Lagerstrom *et al.*, *PCR Methods Applic.* 1:111-19, 1991) and walking PCR (Parker *et al.*, *Nucl. Acids. Res.* 19:3055-60, 1991). Other methods employing amplification may also be employed to obtain a full length cDNA sequence.

In certain instances, it is possible to obtain a full length cDNA sequence by analysis of sequences provided in an expressed sequence tag (EST) database, such as that available from GenBank. Searches for overlapping ESTs may generally be performed using well known programs (*e.g.*, NCBI BLAST searches), and such ESTs may be used to generate a contiguous full length sequence. Full length DNA sequences may also be obtained by analysis of genomic fragments.

20 POLYNUCLEOTIDE EXPRESSION IN HOST CELLS

In other embodiments of the invention, polynucleotide sequences or fragments thereof which encode polypeptides of the invention, or fusion proteins or functional equivalents thereof, may be used in recombinant DNA molecules to direct expression of a polypeptide in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences that encode substantially the same or a functionally equivalent amino acid sequence may be produced and these sequences may be used to clone and express a given polypeptide.

As will be understood by those of skill in the art, it may be advantageous in some instances to produce polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular

prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce a recombinant RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

5 Moreover, the polynucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter polypeptide encoding sequences for a variety of reasons, including but not limited to, alterations which modify the cloning, processing, and/or expression of the gene product. For example, DNA shuffling by random fragmentation and PCR reassembly of gene
10 fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. In addition, site-directed mutagenesis may be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, or introduce mutations, and so forth.

 In another embodiment of the invention, natural, modified, or
15 recombinant nucleic acid sequences may be ligated to a heterologous sequence to encode a fusion protein. For example, to screen peptide libraries for inhibitors of polypeptide activity, it may be useful to encode a chimeric protein that can be recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between the polypeptide-encoding
20 sequence and the heterologous protein sequence, so that the polypeptide may be cleaved and purified away from the heterologous moiety.

 Sequences encoding a desired polypeptide may be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers, M. H. *et al.* (1980) *Nucl. Acids Res. Symp. Ser.* 215-223, Horn, T. *et al.* (1980) *Nucl. Acids Res. Symp. Ser.* 225-232). Alternatively, the protein itself may be produced using chemical
25 methods to synthesize the amino acid sequence of a polypeptide, or a portion thereof. For example, peptide synthesis can be performed using various solid-phase techniques (Roberge, J. Y. *et al.* (1995) *Science* 269:202-204) and automated synthesis may be achieved, for example, using the ABI 431A Peptide Synthesizer (Perkin Elmer, Palo
30 Alto, CA).

A newly synthesized peptide may be substantially purified by preparative high performance liquid chromatography (*e.g.*, Creighton, T. (1983) *Proteins, Structures and Molecular Principles*, WH Freeman and Co., New York, N.Y.) or other comparable techniques available in the art. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing (*e.g.*, the Edman degradation procedure). Additionally, the amino acid sequence of a polypeptide, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

In order to express a desired polypeptide, the nucleotide sequences encoding the polypeptide, or functional equivalents, may be inserted into appropriate expression vector, *i.e.*, a vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding a polypeptide of interest and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook, J. *et al.* (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Plainview, N.Y., and Ausubel, F. M. *et al.* (1989) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y.

A variety of expression vector/host systems may be utilized to contain and express polynucleotide sequences. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus expression vectors (*e.g.*, baculovirus); plant cell systems transformed with virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (*e.g.*, Ti or pBR322 plasmids); or animal cell systems.

The "control elements" or "regulatory sequences" present in an expression vector are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out

transcription and translation. Such elements may vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid
5 lacZ promoter of the PBLUESCRIPT phagemid (Stratagene, La Jolla, Calif.) or PSPORT1 plasmid (Gibco BRL, Gaithersburg, MD) and the like may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are generally preferred. If it is necessary to generate a cell line that contains multiple copies of the sequence encoding a polypeptide, vectors based on SV40 or EBV
10 may be advantageously used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for the expressed polypeptide. For example, when large quantities are needed, for example for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be used.
15 Such vectors include, but are not limited to, the multifunctional *E. coli* cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding the polypeptide of interest may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced; pIN vectors (Van Heeke, G. and S. M. Schuster (1989) *J.*
20 *Biol. Chem.* 264:5503-5509); and the like. pGEX Vectors (Promega, Madison, Wis.) may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems may be designed to
25 include heparin, thrombin, or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel *et al.* (supra) and Grant *et al.* (1987) *Methods*
30 *Enzymol.* 153:516-544.

In cases where plant expression vectors are used, the expression of sequences encoding polypeptides may be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV may be used alone or in combination with the omega leader sequence from TMV (Takamatsu, N. 5 (1987) *EMBO J.* 6:307-311. Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used (Coruzzi, G. *et al.* (1984) *EMBO J.* 3:1671-1680; Broglie, R. *et al.* (1984) *Science* 224:838-843; and Winter, J. *et al.* (1991) *Results Probl. Cell Differ.* 17:85-105). These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. Such techniques 10 are described in a number of generally available reviews (see, for example, Hobbs, S. or Murry, L. E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, N.Y.; pp. 191-196).

An insect system may also be used to express a polypeptide of interest. For example, in one such system, *Autographa californica* nuclear polyhedrosis virus 15 (AcNPV) is used as a vector to express foreign genes in *Spodoptera frugiperda* cells or in *Trichoplusia* larvae. The sequences encoding the polypeptide may be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of the polypeptide-encoding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat 20 protein. The recombinant viruses may then be used to infect, for example, *S. frugiperda* cells or *Trichoplusia* larvae in which the polypeptide of interest may be expressed (Engelhard, E. K. *et al.* (1994) *Proc. Natl. Acad. Sci.* 91 :3224-3227).

In mammalian host cells, a number of viral-based expression systems are generally available. For example, in cases where an adenovirus is used as an expression 25 vector, sequences encoding a polypeptide of interest may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain a viable virus which is capable of expressing the polypeptide in infected host cells (Logan, J. and Shenk, T. (1984) *Proc. Natl. Acad. Sci.* 81:3655-3659). In addition, 30 transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

Specific initiation signals may also be used to achieve more efficient translation of sequences encoding a polypeptide of interest. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding the polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous translational control signals including the ATG initiation codon should be provided. Furthermore, the initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used, such as those described in the literature (Scharf, D. *et al.* (1994) *Results Probl. Cell Differ.* 20:125-162).

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to facilitate correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, HEK293, and WI38, which have specific cellular machinery and characteristic mechanisms for such post-translational activities, may be chosen to ensure the correct modification and processing of the foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is generally preferred. For example, cell lines which stably express a polynucleotide of interest may be transformed using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which

successfully express the introduced sequences. Resistant clones of stably transformed cells may be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler, M. *et al.* (1977) *Cell* 11:223-32) and adenine phosphoribosyltransferase (Lowy, I. *et al.* (1990) *Cell* 22:817-23) genes which can be employed in tk.sup.- or apt.sup.- cells, respectively. Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler, M. *et al.* (1980) *Proc. Natl. Acad. Sci.* 77:3567-70); npt, which confers resistance to the aminoglycosides, neomycin and G-418 (Colbere-Garapin, F. *et al.* (1981) *J. Mol. Biol.* 150:1-14); and als or pat, which confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively (Murry, *supra*). Additional selectable genes have been described, for example, trpB, which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine (Hartman, S. C. and R. C. Mulligan (1988) *Proc. Natl. Acad. Sci.* 85:8047-51). Recently, the use of visible markers has gained popularity with such markers as anthocyanins, beta-glucuronidase and its substrate GUS, and luciferase and its substrate luciferin, being widely used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes, C. A. *et al.* (1995) *Methods Mol. Biol.* 55:121-131).

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression may need to be confirmed. For example, if the sequence encoding a polypeptide is inserted within a marker gene sequence, recombinant cells containing sequences can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a polypeptide-encoding sequence under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

Alternatively, host cells which contain and express a desired polynucleotide sequence may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-

RNA hybridizations and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein.

A variety of protocols for detecting and measuring the expression of polynucleotide-encoded products, using either polyclonal or monoclonal antibodies specific for the product are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on a given polypeptide may be preferred for some applications, but a competitive binding assay may also be employed. These and other assays are described, among other places, in Hampton, R. *et al.* (1990; Serological Methods, a Laboratory Manual, APS Press, St Paul, Minn.) and Maddox, D. E. *et al.* (1983; *J. Exp. Med.* 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides include oligolabeling, nick translation, end-labeling or PCR amplification using a labeled nucleotide. Alternatively, the sequences, or any portions thereof may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits. Suitable reporter molecules or labels, which may be used include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with a polynucleotide sequence of interest may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides of the invention may be designed to contain signal sequences which direct secretion of the

encoded polypeptide through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may be used to join sequences encoding a polypeptide of interest to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor XA or enterokinase (Invitrogen, San Diego, Calif.) between the purification domain and the encoded polypeptide may be used to facilitate purification. One such expression vector provides for expression of a fusion protein containing a polypeptide of interest and a nucleic acid encoding 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography) as described in Porath, J. *et al.* (1992, *Prot. Exp. Purif.* 3:263-281) while the enterokinase cleavage site provides a means for purifying the desired polypeptide from the fusion protein. A discussion of vectors which contain fusion proteins is provided in Kroll, D. J. *et al.* (1993; *DNA Cell Biol.* 12:441-453).

In addition to recombinant production methods, polypeptides of the invention, and fragments thereof, may be produced by direct peptide synthesis using solid-phase techniques (Merrifield J. (1963) *J. Am. Chem. Soc.* 85:2149-2154). Protein synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Alternatively, various fragments may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

SITE-SPECIFIC MUTAGENESIS

Site-specific mutagenesis is a technique useful in the preparation of individual peptides, or biologically functional equivalent polypeptides, through specific mutagenesis of the underlying polynucleotides that encode them. The technique,

well-known to those of skill in the art, further provides a ready ability to prepare and test sequence variants, for example, incorporating one or more of the foregoing considerations, by introducing one or more nucleotide sequence changes into the DNA. Site-specific mutagenesis allows the production of mutants through the use of specific
5 oligonucleotide sequences which encode the DNA sequence of the desired mutation, as well as a sufficient number of adjacent nucleotides, to provide a primer sequence of sufficient size and sequence complexity to form a stable duplex on both sides of the deletion junction being traversed. Mutations may be employed in a selected polynucleotide sequence to improve, alter, decrease, modify, or otherwise change the
10 properties of the polynucleotide itself, and/or alter the properties, activity, composition, stability, or primary sequence of the encoded polypeptide.

In certain embodiments of the present invention, the inventors contemplate the mutagenesis of the disclosed polynucleotide sequences to alter one or more properties of the encoded polypeptide, such as the antigenicity of a polypeptide
15 vaccine. The techniques of site-specific mutagenesis are well-known in the art, and are widely used to create variants of both polypeptides and polynucleotides. For example, site-specific mutagenesis is often used to alter a specific portion of a DNA molecule. In such embodiments, a primer comprising typically about 14 to about 25 nucleotides or so in length is employed, with about 5 to about 10 residues on both sides of the junction of
20 the sequence being altered.

As will be appreciated by those of skill in the art, site-specific mutagenesis techniques have often employed a phage vector that exists in both a single stranded and double stranded form. Typical vectors useful in site-directed mutagenesis include vectors such as the M13 phage. These phage are readily
25 commercially-available and their use is generally well-known to those skilled in the art. Double-stranded plasmids are also routinely employed in site directed mutagenesis that eliminates the step of transferring the gene of interest from a plasmid to a phage.

In general, site-directed mutagenesis in accordance herewith is performed by first obtaining a single-stranded vector or melting apart of two strands of
30 a double-stranded vector that includes within its sequence a DNA sequence that encodes the desired peptide. An oligonucleotide primer bearing the desired mutated

sequence is prepared, generally synthetically. This primer is then annealed with the single-stranded vector, and subjected to DNA polymerizing enzymes such as *E. coli* polymerase I Klenow fragment, in order to complete the synthesis of the mutation-bearing strand. Thus, a heteroduplex is formed wherein one strand encodes the original
5 non-mutated sequence and the second strand bears the desired mutation. This heteroduplex vector is then used to transform appropriate cells, such as *E. coli* cells, and clones are selected which include recombinant vectors bearing the mutated sequence arrangement.

The preparation of sequence variants of the selected peptide-encoding
10 DNA segments using site-directed mutagenesis provides a means of producing potentially useful species and is not meant to be limiting as there are other ways in which sequence variants of peptides and the DNA sequences encoding them may be obtained. For example, recombinant vectors encoding the desired peptide sequence may be treated with mutagenic agents, such as hydroxylamine, to obtain sequence
15 variants. Specific details regarding these methods and protocols are found in the teachings of Maloy *et al.*, 1994; Segal, 1976; Prokop and Bajpai, 1991; Kuby, 1994; and Maniatis *et al.*, 1982, each incorporated herein by reference, for that purpose.

As used herein, the term "oligonucleotide directed mutagenesis procedure" refers to template-dependent processes and vector-mediated propagation
20 which result in an increase in the concentration of a specific nucleic acid molecule relative to its initial concentration, or in an increase in the concentration of a detectable signal, such as amplification. As used herein, the term "oligonucleotide directed mutagenesis procedure" is intended to refer to a process that involves the template-dependent extension of a primer molecule. The term template dependent
25 process refers to nucleic acid synthesis of an RNA or a DNA molecule wherein the sequence of the newly synthesized strand of nucleic acid is dictated by the well-known rules of complementary base pairing (see, for example, Watson, 1987). Typically, vector mediated methodologies involve the introduction of the nucleic acid fragment into a DNA or RNA vector, the clonal amplification of the vector, and the recovery of
30 the amplified nucleic acid fragment. Examples of such methodologies are provided by U. S. Patent No. 4,237,224, specifically incorporated herein by reference in its entirety.

POLYNUCLEOTIDE AMPLIFICATION TECHNIQUES

A number of template dependent processes are available to amplify the target sequences of interest present in a sample. One of the best known amplification methods is the polymerase chain reaction (PCRTM) which is described in detail in U.S. Patent Nos. 4,683,195, 4,683,202 and 4,800,159, each of which is incorporated herein by reference in its entirety. Briefly, in PCRTM, two primer sequences are prepared which are complementary to regions on opposite complementary strands of the target sequence. An excess of deoxynucleoside triphosphates is added to a reaction mixture along with a DNA polymerase (*e.g.*, *Taq* polymerase). If the target sequence is present in a sample, the primers will bind to the target and the polymerase will cause the primers to be extended along the target sequence by adding on nucleotides. By raising and lowering the temperature of the reaction mixture, the extended primers will dissociate from the target to form reaction products, excess primers will bind to the target and to the reaction product and the process is repeated. Preferably reverse transcription and PCRTM amplification procedure may be performed in order to quantify the amount of mRNA amplified. Polymerase chain reaction methodologies are well known in the art.

Another method for amplification is the ligase chain reaction (referred to as LCR), disclosed in Eur. Pat. Appl. Publ. No. 320,308 (specifically incorporated herein by reference in its entirety). In LCR, two complementary probe pairs are prepared, and in the presence of the target sequence, each pair will bind to opposite complementary strands of the target such that they abut. In the presence of a ligase, the two probe pairs will link to form a single unit. By temperature cycling, as in PCRTM, bound ligated units dissociate from the target and then serve as "target sequences" for ligation of excess probe pairs. U.S. Patent No. 4,883,750, incorporated herein by reference in its entirety, describes an alternative method of amplification similar to LCR for binding probe pairs to a target sequence.

Qbeta Replicase, described in PCT Intl. Pat. Appl. Publ. No. PCT/US87/00880, incorporated herein by reference in its entirety, may also be used as still another amplification method in the present invention. In this method, a replicative sequence of RNA that has a region complementary to that of a target is added to a

sample in the presence of an RNA polymerase. The polymerase will copy the replicative sequence that can then be detected.

An isothermal amplification method, in which restriction endonucleases and ligases are used to achieve the amplification of target molecules that contain nucleotide 5'-[α -thio]triphosphates in one strand of a restriction site (Walker *et al.*, 1992, incorporated herein by reference in its entirety), may also be useful in the amplification of nucleic acids in the present invention.

Strand Displacement Amplification (SDA) is another method of carrying out isothermal amplification of nucleic acids which involves multiple rounds of strand displacement and synthesis, *i.e.* nick translation. A similar method, called Repair Chain Reaction (RCR) is another method of amplification which may be useful in the present invention and is involves annealing several probes throughout a region targeted for amplification, followed by a repair reaction in which only two of the four bases are present. The other two bases can be added as biotinylated derivatives for easy detection. A similar approach is used in SDA.

Sequences can also be detected using a cyclic probe reaction (CPR). In CPR, a probe having a 3' and 5' sequences of non-target DNA and an internal or "middle" sequence of the target protein specific RNA is hybridized to DNA which is present in a sample. Upon hybridization, the reaction is treated with RNaseH, and the products of the probe are identified as distinctive products by generating a signal that is released after digestion. The original template is annealed to another cycling probe and the reaction is repeated. Thus, CPR involves amplifying a signal generated by hybridization of a probe to a target gene specific expressed nucleic acid.

Still other amplification methods described in Great Britain Pat. Appl. No. 2 202 328, and in PCT Intl. Pat. Appl. Publ. No. PCT/US89/01025, each of which is incorporated herein by reference in its entirety, may be used in accordance with the present invention. In the former application, "modified" primers are used in a PCR-like, template and enzyme dependent synthesis. The primers may be modified by labeling with a capture moiety (*e.g.*, biotin) and/or a detector moiety (*e.g.*, enzyme). In the latter application, an excess of labeled probes is added to a sample. In the presence of the target sequence, the probe binds and is cleaved catalytically. After cleavage, the

target sequence is released intact to be bound by excess probe. Cleavage of the labeled probe signals the presence of the target sequence.

Other nucleic acid amplification procedures include transcription-based amplification systems (TAS) (Kwoh *et al.*, 1989; PCT Intl. Pat. Appl. Publ. No. WO 88/10315, incorporated herein by reference in its entirety), including nucleic acid
5 sequence based amplification (NASBA) and 3SR. In NASBA, the nucleic acids can be prepared for amplification by standard phenol/chloroform extraction, heat denaturation of a sample, treatment with lysis buffer and minispin columns for isolation of DNA and RNA or guanidinium chloride extraction of RNA. These amplification techniques
10 involve annealing a primer that has sequences specific to the target sequence. Following polymerization, DNA/RNA hybrids are digested with RNase H while double stranded DNA molecules are heat-denatured again. In either case the single stranded DNA is made fully double stranded by addition of second target-specific primer, followed by polymerization. The double stranded DNA molecules are then multiply
15 transcribed by a polymerase such as T7 or SP6. In an isothermal cyclic reaction, the RNAs are reverse transcribed into DNA, and transcribed once again with a polymerase such as T7 or SP6. The resulting products, whether truncated or complete, indicate target-specific sequences.

Eur. Pat. Appl. Publ. No. 329,822, incorporated herein by reference in its
20 entirety, disclose a nucleic acid amplification process involving cyclically synthesizing single-stranded RNA ("ssRNA"), ssDNA, and double-stranded DNA (dsDNA), which may be used in accordance with the present invention. The ssRNA is a first template for a first primer oligonucleotide, which is elongated by reverse transcriptase (RNA-dependent DNA polymerase). The RNA is then removed from resulting
25 DNA:RNA duplex by the action of ribonuclease H (RNase H, an RNase specific for RNA in a duplex with either DNA or RNA). The resultant ssDNA is a second template for a second primer, which also includes the sequences of an RNA polymerase promoter (exemplified by T7 RNA polymerase) 5' to its homology to its template. This primer is then extended by DNA polymerase (exemplified by the large "Klenow"
30 fragment of *E. coli* DNA polymerase I), resulting as a double-stranded DNA ("dsDNA") molecule, having a sequence identical to that of the original RNA between

the primers and having additionally, at one end, a promoter sequence. This promoter sequence can be used by the appropriate RNA polymerase to make many RNA copies of the DNA. These copies can then re-enter the cycle leading to very swift amplification. With proper choice of enzymes, this amplification can be done
5 isothermally without addition of enzymes at each cycle. Because of the cyclical nature of this process, the starting sequence can be chosen to be in the form of either DNA or RNA.

PCT Intl. Pat. Appl. Publ. No. WO 89/06700, incorporated herein by reference in its entirety, disclose a nucleic acid sequence amplification scheme based on
10 the hybridization of a promoter/primer sequence to a target single-stranded DNA ("ssDNA") followed by transcription of many RNA copies of the sequence. This scheme is not cyclic; *i.e.* new templates are not produced from the resultant RNA transcripts. Other amplification methods include "RACE" (Frohman, 1990), and "one-sided PCR" (Ohara, 1989) which are well-known to those of skill in the art.

15 Methods based on ligation of two (or more) oligonucleotides in the presence of nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby amplifying the di-oligonucleotide (Wu and Dean, 1996, incorporated herein by reference in its entirety), may also be used in the amplification of DNA sequences of the present invention.

20 BIOLOGICAL FUNCTIONAL EQUIVALENTS

Modification and changes may be made in the structure of the polynucleotides and polypeptides of the present invention and still obtain a functional molecule that encodes a polypeptide with desirable characteristics. As mentioned above, it is often desirable to introduce one or more mutations into a specific
25 polynucleotide sequence. In certain circumstances, the resulting encoded polypeptide sequence is altered by this mutation, or in other cases, the sequence of the polypeptide is unchanged by one or more mutations in the encoding polynucleotide.

When it is desirable to alter the amino acid sequence of a polypeptide to create an equivalent, or even an improved, second-generation molecule, the amino acid

changes may be achieved by changing one or more of the codons of the encoding DNA sequence, according to Table 1.

For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated by the inventors that various changes may be made in the peptide sequences of the disclosed compositions, or corresponding DNA sequences which encode said peptides without appreciable loss of their biological utility or activity.

TABLE 1

Amino Acids			Codons						
Alanine	Ala	A	GCA	GCC	GCG	GCU			
Cysteine	Cys	C	UGC	UGU					
Aspartic acid	Asp	D	GAC	GAU					
Glutamic acid	Glu	E	GAA	GAG					
Phenylalanine	Phe	F	UUC	UUU					
Glycine	Gly	G	GGA	GGC	GGG	GGU			
Histidine	His	H	CAC	CAU					
Isoleucine	Ile	I	AUA	AUC	AUU				
Lysine	Lys	K	AAA	AAG					
Leucine	Leu	L	UUA	UUG	CUA	CUC	CUG	CUU	
Methionine	Met	M	AUG						
Asparagine	Asn	N	AAC	AAU					
Proline	Pro	P	CCA	CCC	CCG	CCU			
Glutamine	Gln	Q	CAA	CAG					
Arginine	Arg	R	AGA	AGG	CGA	CGC	CGG	CGU	
Serine	Ser	S	AGC	AGU	UCA	UCC	UCG	UCU	
Threonine	Thr	T	ACA	ACC	ACG	ACU			
Valine	Val	V	GUA	GUC	GUG	GUU			
Tryptophan	Trp	W	UGG						
Tyrosine	Tyr	Y	UAC	UAU					

In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of its hydrophobicity and charge characteristics (Kyte and Doolittle, 1982). These values are:

isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (−0.4); threonine (−0.7); serine (−0.8); tryptophan (−0.9); tyrosine (−1.3); proline (−1.6); histidine (−3.2); glutamate (−3.5); glutamine (−3.5); aspartate (−3.5); asparagine (−3.5); lysine (−3.9); and arginine (−4.5).

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, *i.e.* still obtain a biological functionally equivalent protein. In making such changes, the substitution of amino acids whose hydropathic indices are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred. It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. U. S. Patent 4,554,101 (specifically incorporated herein by reference in its entirety), states that the greatest local average hydrophilicity of a protein, as governed by the hydrophilicity of its adjacent amino acids, correlates with a biological property of the protein.

As detailed in U. S. Patent 4,554,101, the following hydrophilicity values have been assigned to amino acid residues: arginine (+3.0); lysine (+3.0); aspartate (+3.0 \pm 1); glutamate (+3.0 \pm 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (−0.4); proline (−0.5 \pm 1); alanine (−0.5); histidine (−0.5); cysteine (−1.0); methionine (−1.3); valine (−1.5); leucine (−1.8); isoleucine (−1.8); tyrosine (−2.3); phenylalanine (−2.5); tryptophan (−3.4). It is understood that an amino acid can be substituted for another having a similar hydrophilicity value and still obtain a biologically equivalent, and in particular, an immunologically equivalent protein. In such changes, the substitution of amino acids whose hydrophilicity values are within ± 2 is preferred, those within ± 1 are particularly preferred, and those within ± 0.5 are even more particularly preferred.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those

of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

In addition, any polynucleotide may be further modified to increase stability *in vivo*. Possible modifications include, but are not limited to, the addition of
5 flanking sequences at the 5' and/or 3' ends; the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages in the backbone; and/or the inclusion of nontraditional bases such as inosine, queosine and wybutosine, as well as acetyl-methyl-, thio- and other modified forms of adenine, cytidine, guanine, thymine and uridine.

10 IN VIVO POLYNUCLEOTIDE DELIVERY TECHNIQUES

In additional embodiments, genetic constructs comprising one or more of the polynucleotides of the invention are introduced into cells *in vivo*. This may be achieved using any of a variety of well known approaches, several of which are outlined below for the purpose of illustration.

15 1. ADENOVIRUS

One of the preferred methods for *in vivo* delivery of one or more nucleic acid sequences involves the use of an adenovirus expression vector. "Adenovirus expression vector" is meant to include those constructs containing adenovirus sequences sufficient to (a) support packaging of the construct and (b) to express a
20 polynucleotide that has been cloned therein in a sense or antisense orientation. Of course, in the context of an antisense construct, expression does not require that the gene product be synthesized.

The expression vector comprises a genetically engineered form of an adenovirus. Knowledge of the genetic organization of adenovirus, a 36 kb, linear,
25 double-stranded DNA virus, allows substitution of large pieces of adenoviral DNA with foreign sequences up to 7 kb (Grunhaus and Horwitz, 1992). In contrast to retrovirus, the adenoviral infection of host cells does not result in chromosomal integration because adenoviral DNA can replicate in an episomal manner without potential genotoxicity. Also, adenoviruses are structurally stable, and no genome rearrangement

has been detected after extensive amplification. Adenovirus can infect virtually all epithelial cells regardless of their cell cycle stage. So far, adenoviral infection appears to be linked only to mild disease such as acute respiratory disease in humans.

Adenovirus is particularly suitable for use as a gene transfer vector because of its mid-sized genome, ease of manipulation, high titer, wide target-cell range and high infectivity. Both ends of the viral genome contain 100-200 base pair inverted repeats (ITRs), which are *cis* elements necessary for viral DNA replication and packaging. The early (E) and late (L) regions of the genome contain different transcription units that are divided by the onset of viral DNA replication. The E1 region (E1A and E1B) encodes proteins responsible for the regulation of transcription of the viral genome and a few cellular genes. The expression of the E2 region (E2A and E2B) results in the synthesis of the proteins for viral DNA replication. These proteins are involved in DNA replication, late gene expression and host cell shut-off (Renan, 1990). The products of the late genes, including the majority of the viral capsid proteins, are expressed only after significant processing of a single primary transcript issued by the major late promoter (MLP). The MLP, (located at 16.8 m.u.) is particularly efficient during the late phase of infection, and all the mRNA's issued from this promoter possess a 5'-tripartite leader (TPL) sequence which makes them preferred mRNA's for translation.

In a current system, recombinant adenovirus is generated from homologous recombination between shuttle vector and provirus vector. Due to the possible recombination between two proviral vectors, wild-type adenovirus may be generated from this process. Therefore, it is critical to isolate a single clone of virus from an individual plaque and examine its genomic structure.

Generation and propagation of the current adenovirus vectors, which are replication deficient, depend on a unique helper cell line, designated 293, which was transformed from human embryonic kidney cells by Ad5 DNA fragments and constitutively expresses E1 proteins (Graham *et al.*, 1977). Since the E3 region is dispensable from the adenovirus genome (Jones and Shenk, 1978), the current adenovirus vectors, with the help of 293 cells, carry foreign DNA in either the E1, the D3 or both regions (Graham and Prevec, 1991). In nature, adenovirus can package

approximately 105% of the wild-type genome (Ghosh-Choudhury *et al.*, 1987), providing capacity for about 2 extra kB of DNA. Combined with the approximately 5.5 kB of DNA that is replaceable in the E1 and E3 regions, the maximum capacity of the current adenovirus vector is under 7.5 kB, or about 15% of the total length of the vector. More than 80% of the adenovirus viral genome remains in the vector backbone and is the source of vector-borne cytotoxicity. Also, the replication deficiency of the E1-deleted virus is incomplete. For example, leakage of viral gene expression has been observed with the currently available vectors at high multiplicities of infection (MOI) (Mulligan, 1993).

10 Helper cell lines may be derived from human cells such as human embryonic kidney cells, muscle cells, hematopoietic cells or other human embryonic mesenchymal or epithelial cells. Alternatively, the helper cells may be derived from the cells of other mammalian species that are permissive for human adenovirus. Such cells include, *e.g.*, Vero cells or other monkey embryonic mesenchymal or epithelial cells.

15 As stated above, the currently preferred helper cell line is 293.

 Recently, Racher *et al.* (1995) disclosed improved methods for culturing 293 cells and propagating adenovirus. In one format, natural cell aggregates are grown by inoculating individual cells into 1 liter siliconized spinner flasks (Techne, Cambridge, UK) containing 100-200 ml of medium. Following stirring at 40 rpm, the cell viability is estimated with trypan blue. In another format, Fibra-Cel microcarriers (Bibby Sterlin, Stone, UK) (5 g/l) is employed as follows. A cell inoculum, resuspended in 5 ml of medium, is added to the carrier (50 ml) in a 250 ml Erlenmeyer flask and left stationary, with occasional agitation, for 1 to 4 h. The medium is then replaced with 50 ml of fresh medium and shaking initiated. For virus production, cells are allowed to grow to about 80% confluence, after which time the medium is replaced (to 25% of the final volume) and adenovirus added at an MOI of 0.05. Cultures are left stationary overnight, following which the volume is increased to 100% and shaking commenced for another 72 h.

 Other than the requirement that the adenovirus vector be replication defective, or at least conditionally defective, the nature of the adenovirus vector is not believed to be crucial to the successful practice of the invention. The adenovirus may

be of any of the 42 different known serotypes or subgroups A-F. Adenovirus type 5 of subgroup C is the preferred starting material in order to obtain a conditional replication-defective adenovirus vector for use in the present invention, since Adenovirus type 5 is a human adenovirus about which a great deal of biochemical and genetic information is known, and it has historically been used for most constructions employing adenovirus as a vector.

As stated above, the typical vector according to the present invention is replication defective and will not have an adenovirus E1 region. Thus, it will be most convenient to introduce the polynucleotide encoding the gene of interest at the position from which the E1-coding sequences have been removed. However, the position of insertion of the construct within the adenovirus sequences is not critical to the invention. The polynucleotide encoding the gene of interest may also be inserted in lieu of the deleted E3 region in E3 replacement vectors as described by Karlsson *et al.* (1986) or in the E4 region where a helper cell line or helper virus complements the E4 defect.

Adenovirus is easy to grow and manipulate and exhibits broad host range *in vitro* and *in vivo*. This group of viruses can be obtained in high titers, *e.g.*, 10^9 - 10^{11} plaque-forming units per ml, and they are highly infective. The life cycle of adenovirus does not require integration into the host cell genome. The foreign genes delivered by adenovirus vectors are episomal and, therefore, have low genotoxicity to host cells. No side effects have been reported in studies of vaccination with wild-type adenovirus (Couch *et al.*, 1963; Top *et al.*, 1971), demonstrating their safety and therapeutic potential as *in vivo* gene transfer vectors.

Adenovirus vectors have been used in eukaryotic gene expression (Levrero *et al.*, 1991; Gomez-Foix *et al.*, 1992) and vaccine development (Grunhaus and Horwitz, 1992; Graham and Prevec, 1992). Recently, animal studies suggested that recombinant adenovirus could be used for gene therapy (Stratford-Perricaudet and Perricaudet, 1991; Stratford-Perricaudet *et al.*, 1990; Rich *et al.*, 1993). Studies in administering recombinant adenovirus to different tissues include trachea instillation (Rosenfeld *et al.*, 1991; Rosenfeld *et al.*, 1992), muscle injection (Ragot *et al.*, 1993),

peripheral intravenous injections (Herz and Gerard, 1993) and stereotactic inoculation into the brain (Le Gal La Salle *et al.*, 1993).

2. RETROVIRUSES

The retroviruses are a group of single-stranded RNA viruses characterized by an ability to convert their RNA to double-stranded DNA in infected cells by a process of reverse-transcription (Coffin, 1990). The resulting DNA then stably integrates into cellular chromosomes as a provirus and directs synthesis of viral proteins. The integration results in the retention of the viral gene sequences in the recipient cell and its descendants. The retroviral genome contains three genes, gag, pol, and env that code for capsid proteins, polymerase enzyme, and envelope components, respectively. A sequence found upstream from the gag gene contains a signal for packaging of the genome into virions. Two long terminal repeat (LTR) sequences are present at the 5' and 3' ends of the viral genome. These contain strong promoter and enhancer sequences and are also required for integration in the host cell genome (Coffin, 1990).

In order to construct a retroviral vector, a nucleic acid encoding one or more oligonucleotide or polynucleotide sequences of interest is inserted into the viral genome in the place of certain viral sequences to produce a virus that is replication-defective. In order to produce virions, a packaging cell line containing the gag, pol, and env genes but without the LTR and packaging components is constructed (Mann *et al.*, 1983). When a recombinant plasmid containing a cDNA, together with the retroviral LTR and packaging sequences is introduced into this cell line (by calcium phosphate precipitation for example), the packaging sequence allows the RNA transcript of the recombinant plasmid to be packaged into viral particles, which are then secreted into the culture media (Nicolas and Rubenstein, 1988; Temin, 1986; Mann *et al.*, 1983). The media containing the recombinant retroviruses is then collected, optionally concentrated, and used for gene transfer. Retroviral vectors are able to infect a broad variety of cell types. However, integration and stable expression require the division of host cells (Paskind *et al.*, 1975).

A novel approach designed to allow specific targeting of retrovirus vectors was recently developed based on the chemical modification of a retrovirus by the chemical addition of lactose residues to the viral envelope. This modification could permit the specific infection of hepatocytes *via* sialoglycoprotein receptors.

5 A different approach to targeting of recombinant retroviruses was designed in which biotinylated antibodies against a retroviral envelope protein and against a specific cell receptor were used. The antibodies were coupled *via* the biotin components by using streptavidin (Roux *et al.*, 1989). Using antibodies against major histocompatibility complex class I and class II antigens, they demonstrated the infection
10 of a variety of human cells that bore those surface antigens with an ecotropic virus *in vitro* (Roux *et al.*, 1989).

3. ADENO-ASSOCIATED VIRUSES

AAV (Ridgeway, 1988; Hermonat and Muzycska, 1984) is a parovirus, discovered as a contamination of adenoviral stocks. It is a ubiquitous virus (antibodies
15 are present in 85% of the US human population) that has not been linked to any disease. It is also classified as a dependovirus, because its replications is dependent on the presence of a helper virus, such as adenovirus. Five serotypes have been isolated, of which AAV-2 is the best characterized. AAV has a single-stranded linear DNA that is encapsidated into capsid proteins VP1, VP2 and VP3 to form an icosahedral virion of
20 20 to 24 nm in diameter (Muzyczka and McLaughlin, 1988).

The AAV DNA is approximately 4.7 kilobases long. It contains two open reading frames and is flanked by two ITRs (FIG. 2). There are two major genes in the AAV genome: *rep* and *cap*. The *rep* gene codes for proteins responsible for viral replications, whereas *cap* codes for capsid protein VP1-3. Each ITR forms a T-shaped
25 hairpin structure. These terminal repeats are the only essential *cis* components of the AAV for chromosomal integration. Therefore, the AAV can be used as a vector with all viral coding sequences removed and replaced by the cassette of genes for delivery. Three viral promoters have been identified and named p5, p19, and p40, according to their map position. Transcription from p5 and p19 results in production of *rep* proteins,

and transcription from p40 produces the capsid proteins (Hermonat and Muzyczka, 1984).

There are several factors that prompted researchers to study the possibility of using rAAV as an expression vector. One is that the requirements for delivering a gene to integrate into the host chromosome are surprisingly few. It is necessary to have the 145-bp ITRs, which are only 6% of the AAV genome. This leaves room in the vector to assemble a 4.5-kb DNA insertion. While this carrying capacity may prevent the AAV from delivering large genes, it is amply suited for delivering the antisense constructs of the present invention.

AAV is also a good choice of delivery vehicles due to its safety. There is a relatively complicated rescue mechanism: not only wild type adenovirus but also AAV genes are required to mobilize rAAV. Likewise, AAV is not pathogenic and not associated with any disease. The removal of viral coding sequences minimizes immune reactions to viral gene expression, and therefore, rAAV does not evoke an inflammatory response.

4. OTHER VIRAL VECTORS AS EXPRESSION CONSTRUCTS

Other viral vectors may be employed as expression constructs in the present invention for the delivery of oligonucleotide or polynucleotide sequences to a host cell. Vectors derived from viruses such as vaccinia virus (Ridgeway, 1988; Coupar *et al.*, 1988), lentiviruses, polio viruses and herpes viruses may be employed. They offer several attractive features for various mammalian cells (Friedmann, 1989; Ridgeway, 1988; Coupar *et al.*, 1988; Horwich *et al.*, 1990).

With the recent recognition of defective hepatitis B viruses, new insight was gained into the structure-function relationship of different viral sequences. *In vitro* studies showed that the virus could retain the ability for helper-dependent packaging and reverse transcription despite the deletion of up to 80% of its genome (Horwich *et al.*, 1990). This suggested that large portions of the genome could be replaced with foreign genetic material. The hepatotropism and persistence (integration) were particularly attractive properties for liver-directed gene transfer. Chang *et al.* (1991) introduced the chloramphenicol acetyltransferase (CAT) gene into duck hepatitis B

virus genome in the place of the polymerase, surface, and pre-surface coding sequences. It was cotransfected with wild-type virus into an avian hepatoma cell line. Culture media containing high titers of the recombinant virus were used to infect primary duckling hepatocytes. Stable CAT gene expression was detected for at least 24 days after transfection (Chang *et al.*, 1991).

5. NON-VIRAL VECTORS

In order to effect expression of the oligonucleotide or polynucleotide sequences of the present invention, the expression construct must be delivered into a cell. This delivery may be accomplished *in vitro*, as in laboratory procedures for transforming cells lines, or *in vivo* or *ex vivo*, as in the treatment of certain disease states. As described above, one preferred mechanism for delivery is *via* viral infection where the expression construct is encapsulated in an infectious viral particle.

Once the expression construct has been delivered into the cell the nucleic acid encoding the desired oligonucleotide or polynucleotide sequences may be positioned and expressed at different sites. In certain embodiments, the nucleic acid encoding the construct may be stably integrated into the genome of the cell. This integration may be in the specific location and orientation *via* homologous recombination (gene replacement) or it may be integrated in a random, non-specific location (gene augmentation). In yet further embodiments, the nucleic acid may be stably maintained in the cell as a separate, episomal segment of DNA. Such nucleic acid segments or "episomes" encode sequences sufficient to permit maintenance and replication independent of or in synchronization with the host cell cycle. How the expression construct is delivered to a cell and where in the cell the nucleic acid remains is dependent on the type of expression construct employed.

In certain embodiments of the invention, the expression construct comprising one or more oligonucleotide or polynucleotide sequences may simply consist of naked recombinant DNA or plasmids. Transfer of the construct may be performed by any of the methods mentioned above which physically or chemically permeabilize the cell membrane. This is particularly applicable for transfer *in vitro* but it may be applied to *in vivo* use as well. Dubensky *et al.* (1984) successfully injected

polyomavirus DNA in the form of calcium phosphate precipitates into liver and spleen of adult and newborn mice demonstrating active viral replication and acute infection. Benvenisty and Reshef (1986) also demonstrated that direct intraperitoneal injection of calcium phosphate-precipitated plasmids results in expression of the transfected genes.

- 5 It is envisioned that DNA encoding a gene of interest may also be transferred in a similar manner *in vivo* and express the gene product.

Another embodiment of the invention for transferring a naked DNA expression construct into cells may involve particle bombardment. This method depends on the ability to accelerate DNA-coated microprojectiles to a high velocity
10 allowing them to pierce cell membranes and enter cells without killing them (Klein *et al.*, 1987). Several devices for accelerating small particles have been developed. One such device relies on a high voltage discharge to generate an electrical current, which in turn provides the motive force (Yang *et al.*, 1990). The microprojectiles used have consisted of biologically inert substances such as tungsten or gold beads.

- 15 Selected organs including the liver, skin, and muscle tissue of rats and mice have been bombarded *in vivo* (Yang *et al.*, 1990; Zelenin *et al.*, 1991). This may require surgical exposure of the tissue or cells, to eliminate any intervening tissue between the gun and the target organ, *i.e. ex vivo* treatment. Again, DNA encoding a particular gene may be delivered *via* this method and still be incorporated by the present
20 invention.

ANTISENSE OLIGONUCLEOTIDES

- The end result of the flow of genetic information is the synthesis of protein. DNA is transcribed by polymerases into messenger RNA and translated on the ribosome to yield a folded, functional protein. Thus there are several steps along the
25 route where protein synthesis can be inhibited. The native DNA segment coding for a polypeptide described herein, as all such mammalian DNA strands, has two strands: a sense strand and an antisense strand held together by hydrogen bonding. The messenger RNA coding for polypeptide has the same nucleotide sequence as the sense DNA strand except that the DNA thymidine is replaced by uridine. Thus, synthetic

antisense nucleotide sequences will bind to a mRNA and inhibit expression of the protein encoded by that mRNA.

The targeting of antisense oligonucleotides to mRNA is thus one mechanism to shut down protein synthesis, and, consequently, represents a powerful and targeted therapeutic approach. For example, the synthesis of polygalacturonase and the muscarine type 2 acetylcholine receptor are inhibited by antisense oligonucleotides directed to their respective mRNA sequences (U. S. Patent 5,739,119 and U. S. Patent 5,759,829, each specifically incorporated herein by reference in its entirety). Further, examples of antisense inhibition have been demonstrated with the nuclear protein cyclin, the multiple drug resistance gene (MDG1), ICAM-1, E-selectin, STK-1, striatal GABA_A receptor and human EGF (Jaskulski *et al.*, 1988; Vasanthakumar and Ahmed, 1989; Peris *et al.*, 1998; U. S. Patent 5,801,154; U. S. Patent 5,789,573; U. S. Patent 5,718,709 and U. S. Patent 5,610,288, each specifically incorporated herein by reference in its entirety). Antisense constructs have also been described that inhibit and can be used to treat a variety of abnormal cellular proliferations, *e.g.* cancer (U. S. Patent 5,747,470; U. S. Patent 5,591,317 and U. S. Patent 5,783,683, each specifically incorporated herein by reference in its entirety).

Therefore, in exemplary embodiments, the invention provides oligonucleotide sequences that comprise all, or a portion of, any sequence that is capable of specifically binding to polynucleotide sequence described herein, or a complement thereof. In one embodiment, the antisense oligonucleotides comprise DNA or derivatives thereof. In another embodiment, the oligonucleotides comprise RNA or derivatives thereof. In a third embodiment, the oligonucleotides are modified DNAs comprising a phosphorothioated modified backbone. In a fourth embodiment, the oligonucleotide sequences comprise peptide nucleic acids or derivatives thereof. In each case, preferred compositions comprise a sequence region that is complementary, and more preferably substantially-complementary, and even more preferably, completely complementary to one or more portions of polynucleotides disclosed herein.

Selection of antisense compositions specific for a given gene sequence is based upon analysis of the chosen target sequence (*i.e.* in these illustrative examples the rat and human sequences) and determination of secondary structure, T_m , binding

energy, relative stability, and antisense compositions were selected based upon their relative inability to form dimers, hairpins, or other secondary structures that would reduce or prohibit specific binding to the target mRNA in a host cell.

Highly preferred target regions of the mRNA, are those which are at or
5 near the AUG translation initiation codon, and those sequences which were substantially complementary to 5' regions of the mRNA. These secondary structure analyses and target site selection considerations were performed using v.4 of the OLIGO primer analysis software (Rychlik, 1997) and the BLASTN 2.0.5 algorithm software (Altschul *et al.*, 1997).

10 The use of an antisense delivery method employing a short peptide vector, termed MPG (27 residues), is also contemplated. The MPG peptide contains a hydrophobic domain derived from the fusion sequence of HIV gp41 and a hydrophilic domain from the nuclear localization sequence of SV40 T-antigen (Morris *et al.*, 1997). It has been demonstrated that several molecules of the MPG peptide coat the antisense
15 oligonucleotides and can be delivered into cultured mammalian cells in less than 1 hour with relatively high efficiency (90%). Further, the interaction with MPG strongly increases both the stability of the oligonucleotide to nuclease and the ability to cross the plasma membrane (Morris *et al.*, 1997).

RIBOZYMES

20 Although proteins traditionally have been used for catalysis of nucleic acids, another class of macromolecules has emerged as useful in this endeavor. Ribozymes are RNA-protein complexes that cleave nucleic acids in a site-specific fashion. Ribozymes have specific catalytic domains that possess endonuclease activity (Kim and Cech, 1987; Gerlach *et al.*, 1987; Forster and Symons, 1987). For example, a
25 large number of ribozymes accelerate phosphoester transfer reactions with a high degree of specificity, often cleaving only one of several phosphoesters in an oligonucleotide substrate (Cech *et al.*, 1981; Michel and Westhof, 1990; Reinhold-Hurek and Shub, 1992). This specificity has been attributed to the requirement that the substrate bind via specific base-pairing interactions to the internal guide sequence
30 ("IGS") of the ribozyme prior to chemical reaction.

Ribozyme catalysis has primarily been observed as part of sequence-specific cleavage/ligation reactions involving nucleic acids (Joyce, 1989; Cech *et al.*, 1981). For example, U. S. Patent No. 5,354,855 (specifically incorporated herein by reference) reports that certain ribozymes can act as endonucleases with a sequence
5 specificity greater than that of known ribonucleases and approaching that of the DNA restriction enzymes. Thus, sequence-specific ribozyme-mediated inhibition of gene expression may be particularly suited to therapeutic applications (Scanlon *et al.*, 1991; Sarver *et al.*, 1990). Recently, it was reported that ribozymes elicited genetic changes in some cells lines to which they were applied; the altered genes included the oncogenes
10 *H-ras*, *c-fos* and genes of HIV. Most of this work involved the modification of a target mRNA, based on a specific mutant codon that is cleaved by a specific ribozyme.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds *in trans* (and thus can cleave other RNA molecules) under physiological conditions. In general,
15 enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to
20 cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over many
25 technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of
30 target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target

RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not prevent their action (Woolf *et al.*, 1992). Thus, the specificity of action of a ribozyme is greater than that of
5 an antisense oligonucleotide binding the same RNA site.

The enzymatic nucleic acid molecule may be formed in a hammerhead, hairpin, a hepatitis δ virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or Neurospora VS RNA motif. Examples of hammerhead motifs are described by Rossi *et al.* (1992). Examples of hairpin motifs are described by Hampel
10 *et al.* (Eur. Pat. Appl. Publ. No. EP 0360257), Hampel and Tritz (1989), Hampel *et al.* (1990) and U. S. Patent 5,631,359 (specifically incorporated herein by reference). An example of the hepatitis δ virus motif is described by Perrotta and Been (1992); an example of the RNaseP motif is described by Guerrier-Takada *et al.* (1983); Neurospora VS RNA ribozyme motif is described by Collins (Saville and Collins,
15 1990; Saville and Collins, 1991; Collins and Olive, 1993); and an example of the Group I intron is described in (U. S. Patent 4,987,071, specifically incorporated herein by reference). All that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or
20 surrounding that substrate binding site which impart an RNA cleaving activity to the molecule. Thus the ribozyme constructs need not be limited to specific motifs mentioned herein.

In certain embodiments, it may be important to produce enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired
25 target, such as one of the sequences disclosed herein. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNA. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

30 Small enzymatic nucleic acid motifs (*e.g.*, of the hammerhead or the hairpin structure) may also be used for exogenous delivery. The simple structure of

these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. Alternatively, catalytic RNA molecules can be expressed within cells from eukaryotic promoters (*e.g.*, Scanlon *et al.*, 1991; Kashani-Sabet *et al.*, 1992; Dropulic *et al.*, 1992; Weerasinghe *et al.*, 1991; Ojwang *et al.*, 1992; 5 Chen *et al.*, 1992; Sarver *et al.*, 1990). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Int. Pat. Appl. Publ. No. WO 93/23569, and Int. Pat. Appl. Publ. No. WO 94/02595, both hereby incorporated by reference; Ohkawa *et al.*, 10 1992; Taira *et al.*, 1991; and Ventura *et al.*, 1993).

Ribozymes may be added directly, or can be complexed with cationic lipids, lipid complexes, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues *ex vivo*, or *in vivo* through injection, aerosol inhalation, infusion pump or stent, with or 15 without their incorporation in biopolymers.

Ribozymes may be designed as described in Int. Pat. Appl. Publ. No. WO 93/23569 and Int. Pat. Appl. Publ. No. WO 94/02595, each specifically incorporated herein by reference) and synthesized to be tested *in vitro* and *in vivo*, as described. Such ribozymes can also be optimized for delivery. While specific 20 examples are provided, those in the art will recognize that equivalent RNA targets in other species can be utilized when necessary.

Hammerhead or hairpin ribozymes may be individually analyzed by computer folding (Jaeger *et al.*, 1989) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable 25 intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 or so bases on each arm are able to bind to, or otherwise interact with, the target RNA.

Ribozymes of the hammerhead or hairpin motif may be designed to 30 anneal to various sites in the mRNA message, and can be chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described

in Usman *et al.* (1987) and in Scaringe *et al.* (1990) and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. Average stepwise coupling yields are typically >98%. Hairpin ribozymes may be synthesized in two parts and annealed to reconstruct an active ribozyme (Chowrira and Burke, 1992). Ribozymes may be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-o-methyl, 2'-H (for a review see *e.g.*, Usman and Cedergren, 1992). Ribozymes may be purified by gel electrophoresis using general methods or by high pressure liquid chromatography and resuspended in water.

Ribozyme activity can be optimized by altering the length of the ribozyme binding arms, or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Int. Pat. Appl. Publ. No. WO 92/07065; Perrault *et al.*, 1990; Picken *et al.*, 1991; Usman and Cedergren, 1992; Int. Pat. Appl. Publ. No. WO 93/15187; Int. Pat. Appl. Publ. No. WO 91/03162; Eur. Pat. Appl. Publ. No. 92110298.4; U. S. Patent 5,334,711; and Int. Pat. Appl. Publ. No. WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan *et al.* (Int. Pat. Appl. Publ. No. WO 94/02595) describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination may be locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions

of ribozyme delivery and administration are provided in Int. Pat. Appl. Publ. No. WO 94/02595 and Int. Pat. Appl. Publ. No. WO 93/23569, each specifically incorporated herein by reference.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, *etc.*) present nearby. Prokaryotic RNA polymerase promoters may also be used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990; Gao and Huang, 1993; Lieber *et al.*, 1993; Zhou *et al.*, 1990). Ribozymes expressed from such promoters can function in mammalian cells (*e.g.* Kashani-Saber *et al.*, 1992; Ojwang *et al.*, 1992; Chen *et al.*, 1992; Yu *et al.*, 1993; L'Huillier *et al.*, 1992; Lisiewicz *et al.*, 1993). Such transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated vectors), or viral RNA vectors (such as retroviral, semliki forest virus, sindbis virus vectors).

Ribozymes may be used as diagnostic tools to examine genetic drift and mutations within diseased cells. They can also be used to assess levels of the target RNA molecule. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These studies will lead to better treatment of the disease progression by affording the possibility of combinational

therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes are well known in the art, and include detection of the presence of mRNA associated with an IL-5 related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

PEPTIDE NUCLEIC ACIDS

In certain embodiments, the inventors contemplate the use of peptide nucleic acids (PNAs) in the practice of the methods of the invention. PNA is a DNA mimic in which the nucleobases are attached to a pseudopeptide backbone (Good and Nielsen, 1997). PNA is able to be utilized in a number methods that traditionally have used RNA or DNA. Often PNA sequences perform better in techniques than the corresponding RNA or DNA sequences and have utilities that are not inherent to RNA or DNA. A review of PNA including methods of making, characteristics of, and methods of using, is provided by Corey (1997) and is incorporated herein by reference. As such, in certain embodiments, one may prepare PNA sequences that are complementary to one or more portions of the ACE mRNA sequence, and such PNA compositions may be used to regulate, alter, decrease, or reduce the translation of ACE-specific mRNA, and thereby alter the level of ACE activity in a host cell to which such PNA compositions have been administered.

PNAs have 2-aminoethyl-glycine linkages replacing the normal phosphodiester backbone of DNA (Nielsen *et al.*, 1991; Hanvey *et al.*, 1992; Hyrup and Nielsen, 1996; Neilsen, 1996). This chemistry has three important consequences: firstly, in contrast to DNA or phosphorothioate oligonucleotides, PNAs are neutral molecules; secondly, PNAs are achiral, which avoids the need to develop a stereoselective synthesis; and thirdly, PNA synthesis uses standard Boc (Dueholm *et al.*, 1994) or Fmoc (Thomson *et al.*, 1995) protocols for solid-phase peptide synthesis, although other methods, including a modified Merrifield method, have been used (Christensen *et al.*, 1995).

PNA monomers or ready-made oligomers are commercially available from PerSeptive Biosystems (Framingham, MA). PNA syntheses by either Boc or Fmoc protocols are straightforward using manual or automated protocols (Norton *et al.*, 1995). The manual protocol lends itself to the production of chemically modified PNAs or the simultaneous synthesis of families of closely related PNAs.

As with peptide synthesis, the success of a particular PNA synthesis will depend on the properties of the chosen sequence. For example, while in theory PNAs can incorporate any combination of nucleotide bases, the presence of adjacent purines can lead to deletions of one or more residues in the product. In expectation of this difficulty, it is suggested that, in producing PNAs with adjacent purines, one should repeat the coupling of residues likely to be added inefficiently. This should be followed by the purification of PNAs by reverse-phase high-pressure liquid chromatography (Norton *et al.*, 1995) providing yields and purity of product similar to those observed during the synthesis of peptides.

Modifications of PNAs for a given application may be accomplished by coupling amino acids during solid-phase synthesis or by attaching compounds that contain a carboxylic acid group to the exposed N-terminal amine. Alternatively, PNAs can be modified after synthesis by coupling to an introduced lysine or cysteine. The ease with which PNAs can be modified facilitates optimization for better solubility or for specific functional requirements. Once synthesized, the identity of PNAs and their derivatives can be confirmed by mass spectrometry. Several studies have made and utilized modifications of PNAs (Norton *et al.*, 1995; Haaima *et al.*, 1996; Stetsenko *et al.*, 1996; Petersen *et al.*, 1995; Ulmann *et al.*, 1996; Koch *et al.*, 1995; Orum *et al.*, 1995; Footer *et al.*, 1996; Griffith *et al.*, 1995; Kremsky *et al.*, 1996; Pardridge *et al.*, 1995; Boffa *et al.*, 1995; Landsdorp *et al.*, 1996; Gambacorti-Passerini *et al.*, 1996; Armitage *et al.*, 1997; Seeger *et al.*, 1997; Ruskowski *et al.*, 1997). U.S. Patent No. 5,700,922 discusses PNA-DNA-PNA chimeric molecules and their uses in diagnostics, modulating protein in organisms, and treatment of conditions susceptible to therapeutics.

In contrast to DNA and RNA, which contain negatively charged linkages, the PNA backbone is neutral. In spite of this dramatic alteration, PNAs

recognize complementary DNA and RNA by Watson-Crick pairing (Egholm *et al.*, 1993), validating the initial modeling by Nielsen *et al.* (1991). PNAs lack 3' to 5' polarity and can bind in either parallel or antiparallel fashion, with the antiparallel mode being preferred (Egholm *et al.*, 1993).

5 Hybridization of DNA oligonucleotides to DNA and RNA is destabilized by electrostatic repulsion between the negatively charged phosphate backbones of the complementary strands. By contrast, the absence of charge repulsion in PNA-DNA or PNA-RNA duplexes increases the melting temperature (T_m) and reduces the dependence of T_m on the concentration of mono- or divalent cations
10 (Nielsen *et al.*, 1991). The enhanced rate and affinity of hybridization are significant because they are responsible for the surprising ability of PNAs to perform strand invasion of complementary sequences within relaxed double-stranded DNA. In addition, the efficient hybridization at inverted repeats suggests that PNAs can recognize secondary structure effectively within double-stranded DNA. Enhanced
15 recognition also occurs with PNAs immobilized on surfaces, and Wang *et al.* have shown that support-bound PNAs can be used to detect hybridization events (Wang *et al.*, 1996).

One might expect that tight binding of PNAs to complementary sequences would also increase binding to similar (but not identical) sequences, reducing
20 the sequence specificity of PNA recognition. As with DNA hybridization, however, selective recognition can be achieved by balancing oligomer length and incubation temperature. Moreover, selective hybridization of PNAs is encouraged by PNA-DNA hybridization being less tolerant of base mismatches than DNA-DNA hybridization. For example, a single mismatch within a 16 bp PNA-DNA duplex can reduce the T_m by
25 up to 15°C (Egholm *et al.*, 1993). This high level of discrimination has allowed the development of several PNA-based strategies for the analysis of point mutations (Wang *et al.*, 1996; Carlsson *et al.*, 1996; Thiede *et al.*, 1996; Webb and Hurskainen, 1996; Perry-O'Keefe *et al.*, 1996).

High-affinity binding provides clear advantages for molecular
30 recognition and the development of new applications for PNAs. For example, 11-13 nucleotide PNAs inhibit the activity of telomerase, a ribonucleo-protein that extends

telomere ends using an essential RNA template, while the analogous DNA oligomers do not (Norton *et al.*, 1996).

Neutral PNAs are more hydrophobic than analogous DNA oligomers, and this can lead to difficulty solubilizing them at neutral pH, especially if the PNAs have a high purine content or if they have the potential to form secondary structures. Their solubility can be enhanced by attaching one or more positive charges to the PNA termini (Nielsen *et al.*, 1991).

Findings by Allfrey and colleagues suggest that strand invasion will occur spontaneously at sequences within chromosomal DNA (Boffa *et al.*, 1995; Boffa *et al.*, 1996). These studies targeted PNAs to triplet repeats of the nucleotides CAG and used this recognition to purify transcriptionally active DNA (Boffa *et al.*, 1995) and to inhibit transcription (Boffa *et al.*, 1996). This result suggests that if PNAs can be delivered within cells then they will have the potential to be general sequence-specific regulators of gene expression. Studies and reviews concerning the use of PNAs as antisense and anti-gene agents include Nielsen *et al.* (1993b), Hanvey *et al.* (1992), and Good and Nielsen (1997). Koppelhus *et al.* (1997) have used PNAs to inhibit HIV-1 inverse transcription, showing that PNAs may be used for antiviral therapies.

Methods of characterizing the antisense binding properties of PNAs are discussed in Rose (1993) and Jensen *et al.* (1997). Rose uses capillary gel electrophoresis to determine binding of PNAs to their complementary oligonucleotide, measuring the relative binding kinetics and stoichiometry. Similar types of measurements were made by Jensen *et al.* using BIAcore™ technology.

Other applications of PNAs include use in DNA strand invasion (Nielsen *et al.*, 1991), antisense inhibition (Hanvey *et al.*, 1992), mutational analysis (Orum *et al.*, 1993), enhancers of transcription (Mollegaard *et al.*, 1994), nucleic acid purification (Orum *et al.*, 1995), isolation of transcriptionally active genes (Boffa *et al.*, 1995), blocking of transcription factor binding (Vickers *et al.*, 1995), genome cleavage (Veselkov *et al.*, 1996), biosensors (Wang *et al.*, 1996), *in situ* hybridization (Thisted *et al.*, 1996), and in a alternative to Southern blotting (Perry-O'Keefe, 1996).

POLYPEPTIDE COMPOSITIONS

The present invention, in other aspects, provides polypeptide compositions. Generally, a polypeptide of the invention will be an isolated polypeptide (or an epitope, variant, or active fragment thereof) derived from a mammalian species.

5 Preferably, the polypeptide is encoded by a polynucleotide sequence disclosed herein or a sequence which hybridizes under moderately stringent conditions to a polynucleotide sequence disclosed herein. Alternatively, the polypeptide may be defined as a polypeptide which comprises a contiguous amino acid sequence from an amino acid sequence disclosed herein, or which polypeptide comprises an entire amino acid
10 sequence disclosed herein.

In the present invention, a polypeptide composition is also understood to comprise one or more polypeptides that are immunologically reactive with antibodies generated against a polypeptide of the invention, particularly a polypeptide having the amino acid sequence disclosed in SEQ ID NO:126, or to active fragments, or to variants
15 or biological functional equivalents thereof.

Likewise, a polypeptide composition of the present invention is understood to comprise one or more polypeptides that are capable of eliciting antibodies that are immunologically reactive with one or more polypeptides encoded by one or more contiguous nucleic acid sequences contained in SEQ ID NO:1-125, or to active
20 fragments, or to variants thereof, or to one or more nucleic acid sequences which hybridize to one or more of these sequences under conditions of moderate to high stringency. Particularly illustrative polypeptides include the amino acid sequence disclosed in SEQ ID NO:126.

As used herein, an active fragment of a polypeptide includes a whole or
25 a portion of a polypeptide which is modified by conventional techniques, *e.g.*, mutagenesis, or by addition, deletion, or substitution, but which active fragment exhibits substantially the same structure function, antigenicity, etc., as a polypeptide as described herein.

In certain illustrative embodiments, the polypeptides of the invention
30 will comprise at least an immunogenic portion of a breast tumor protein or a variant thereof, as described herein. As noted above, a "breast tumor protein" is a protein that

is expressed by breast tumor cells. Proteins that are breast tumor proteins also react detectably within an immunoassay (such as an ELISA) with antisera from a patient with breast cancer. Polypeptides as described herein may be of any length. Additional sequences derived from the native protein and/or heterologous sequences may be present, and such sequences may (but need not) possess further immunogenic or antigenic properties.

An "immunogenic portion," as used herein is a portion of a protein that is recognized (*i.e.*, specifically bound) by a B-cell and/or T-cell surface antigen receptor. Such immunogenic portions generally comprise at least 5 amino acid residues, more preferably at least 10, and still more preferably at least 20 amino acid residues of a breast tumor protein or a variant thereof. Certain preferred immunogenic portions include peptides in which an N-terminal leader sequence and/or transmembrane domain have been deleted. Other preferred immunogenic portions may contain a small N- and/or C-terminal deletion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids), relative to the mature protein.

Immunogenic portions may generally be identified using well known techniques, such as those summarized in Paul, *Fundamental Immunology*, 3rd ed., 243-247 (Raven Press, 1993) and references cited therein. Such techniques include screening polypeptides for the ability to react with antigen-specific antibodies, antisera and/or T-cell lines or clones. As used herein, antisera and antibodies are "antigen-specific" if they specifically bind to an antigen (*i.e.*, they react with the protein in an ELISA or other immunoassay, and do not react detectably with unrelated proteins). Such antisera and antibodies may be prepared as described herein, and using well known techniques. An immunogenic portion of a native breast tumor protein is a portion that reacts with such antisera and/or T-cells at a level that is not substantially less than the reactivity of the full length polypeptide (*e.g.*, in an ELISA and/or T-cell reactivity assay). Such immunogenic portions may react within such assays at a level that is similar to or greater than the reactivity of the full length polypeptide. Such screens may generally be performed using methods well known to those of ordinary skill in the art, such as those described in Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. For example, a polypeptide may be

immobilized on a solid support and contacted with patient sera to allow binding of antibodies within the sera to the immobilized polypeptide. Unbound sera may then be removed and bound antibodies detected using, for example, ¹²⁵I-labeled Protein A.

As noted above, a composition may comprise a variant of a native breast tumor protein. A polypeptide "variant," as used herein, is a polypeptide that differs from a native breast tumor protein in one or more substitutions, deletions, additions and/or insertions, such that the immunogenicity of the polypeptide is not substantially diminished. In other words, the ability of a variant to react with antigen-specific antisera may be enhanced or unchanged, relative to the native protein, or may be diminished by less than 50%, and preferably less than 20%, relative to the native protein. Such variants may generally be identified by modifying one of the above polypeptide sequences and evaluating the reactivity of the modified polypeptide with antigen-specific antibodies or antisera as described herein. Preferred variants include those in which one or more portions, such as an N-terminal leader sequence or transmembrane domain, have been removed. Other preferred variants include variants in which a small portion (*e.g.*, 1-30 amino acids, preferably 5-15 amino acids) has been removed from the N- and/or C-terminal of the mature protein.

Polypeptide variants encompassed by the present invention include those exhibiting at least about 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% or more identity (determined as described above) to the polypeptides disclosed herein.

Preferably, a variant contains conservative substitutions. A "conservative substitution" is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and hydropathic nature of the polypeptide to be substantially unchanged. Amino acid substitutions may generally be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity and/or the amphipathic nature of the residues. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine and valine; glycine and

alanine; asparagine and glutamine; and serine, threonine, phenylalanine and tyrosine. Other groups of amino acids that may represent conservative changes include: (1) ala, pro, gly, glu, asp, gln, asn, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his. A variant may also, or alternatively, contain nonconservative changes. In a preferred embodiment, variant polypeptides differ from a native sequence by substitution, deletion or addition of five amino acids or fewer. Variants may also (or alternatively) be modified by, for example, the deletion or addition of amino acids that have minimal influence on the immunogenicity, secondary structure and hydrophobic nature of the polypeptide.

As noted above, polypeptides may comprise a signal (or leader) sequence at the N-terminal end of the protein, which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification or identification of the polypeptide (e.g., poly-His), or to enhance binding of the polypeptide to a solid support.

For example, a polypeptide may be conjugated to an immunoglobulin Fc region.

Polypeptides may be prepared using any of a variety of well known techniques. Recombinant polypeptides encoded by DNA sequences as described above may be readily prepared from the DNA sequences using any of a variety of expression vectors known to those of ordinary skill in the art. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a DNA molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, and higher eukaryotic cells, such as mammalian cells and plant cells. Preferably, the host cells employed are *E. coli*, yeast or a mammalian cell line such as COS or CHO. Supernatants from suitable host/vector systems which secrete recombinant protein or polypeptide into culture media may be first concentrated using a commercially available filter. Following concentration, the concentrate may be applied to a suitable purification matrix such as an affinity matrix or an ion exchange resin. Finally, one or more reverse phase HPLC steps can be employed to further purify a recombinant polypeptide.

Portions and other variants having less than about 100 amino acids, and generally less than about 50 amino acids, may also be generated by synthetic means,

using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. *See Merrifield, J. Am. Chem. Soc.* 5 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied BioSystems Division (Foster City, CA), and may be operated according to the manufacturer's instructions.

Within certain specific embodiments, a polypeptide may be a fusion 10 protein that comprises multiple polypeptides as described herein, or that comprises at least one polypeptide as described herein and an unrelated sequence, such as a known tumor protein. A fusion partner may, for example, assist in providing T helper epitopes (an immunological fusion partner), preferably T helper epitopes recognized by humans, or may assist in expressing the protein (an expression enhancer) at higher yields than 15 the native recombinant protein. Certain preferred fusion partners are both immunological and expression enhancing fusion partners. Other fusion partners may be selected so as to increase the solubility of the protein or to enable the protein to be targeted to desired intracellular compartments. Still further fusion partners include affinity tags, which facilitate purification of the protein.

20 Fusion proteins may generally be prepared using standard techniques, including chemical conjugation. Preferably, a fusion protein is expressed as a recombinant protein, allowing the production of increased levels, relative to a non-fused protein, in an expression system. Briefly, DNA sequences encoding the polypeptide components may be assembled separately, and ligated into an appropriate expression 25 vector. The 3' end of the DNA sequence encoding one polypeptide component is ligated, with or without a peptide linker, to the 5' end of a DNA sequence encoding the second polypeptide component so that the reading frames of the sequences are in phase. This permits translation into a single fusion protein that retains the biological activity of both component polypeptides.

30 A peptide linker sequence may be employed to separate the first and second polypeptide components by a distance sufficient to ensure that each polypeptide

5 folds into its secondary and tertiary structures. Such a peptide linker sequence is incorporated into the fusion protein using standard techniques well known in the art. Suitable peptide linker sequences may be chosen based on the following factors: (1) their ability to adopt a flexible extended conformation; (2) their inability to adopt a
10 secondary structure that could interact with functional epitopes on the first and second polypeptides; and (3) the lack of hydrophobic or charged residues that might react with the polypeptide functional epitopes. Preferred peptide linker sequences contain Gly, Asn and Ser residues. Other near neutral amino acids, such as Thr and Ala may also be used in the linker sequence. Amino acid sequences which may be usefully employed as
15 linkers include those disclosed in Maratea *et al.*, *Gene* 40:39-46, 1985; Murphy *et al.*, *Proc. Natl. Acad. Sci. USA* 83:8258-8262, 1986; U.S. Patent No. 4,935,233 and U.S. Patent No. 4,751,180. The linker sequence may generally be from 1 to about 50 amino acids in length. Linker sequences are not required when the first and second polypeptides have non-essential N-terminal amino acid regions that can be used to
20 separate the functional domains and prevent steric interference.

The ligated DNA sequences are operably linked to suitable transcriptional or translational regulatory elements. The regulatory elements responsible for expression of DNA are located only 5' to the DNA sequence encoding the first polypeptides. Similarly, stop codons required to end translation and
25 transcription termination signals are only present 3' to the DNA sequence encoding the second polypeptide.

Fusion proteins are also provided. Such proteins comprise a polypeptide as described herein together with an unrelated immunogenic protein. Preferably the immunogenic protein is capable of eliciting a recall response. Examples of such
30 proteins include tetanus, tuberculosis and hepatitis proteins (*see*, for example, Stoute *et al. New Engl. J. Med.*, 336:86-91, 1997).

Within preferred embodiments, an immunological fusion partner is derived from protein D, a surface protein of the gram-negative bacterium *Haemophilus influenza B* (WO 91/18926). Preferably, a protein D derivative comprises
35 approximately the first third of the protein (*e.g.*, the first N-terminal 100-110 amino acids), and a protein D derivative may be lipidated. Within certain preferred

embodiments, the first 109 residues of a Lipoprotein D fusion partner is included on the N-terminus to provide the polypeptide with additional exogenous T-cell epitopes and to increase the expression level in *E. coli* (thus functioning as an expression enhancer). The lipid tail ensures optimal presentation of the antigen to antigen presenting cells.

- 5 Other fusion partners include the non-structural protein from influenzae virus, NS1 (hemagglutinin). Typically, the N-terminal 81 amino acids are used, although different fragments that include T-helper epitopes may be used.

In another embodiment, the immunological fusion partner is the protein known as LYTA, or a portion thereof (preferably a C-terminal portion). LYTA is derived from *Streptococcus pneumoniae*, which synthesizes an N-acetyl-L-alanine
10 amidase known as amidase LYTA (encoded by the *LytA* gene; *Gene* 43:265-292, 1986). LYTA is an autolysin that specifically degrades certain bonds in the peptidoglycan backbone. The C-terminal domain of the LYTA protein is responsible for the affinity to the choline or to some choline analogues such as DEAE. This
15 property has been exploited for the development of *E. coli* C-LYTA expressing plasmids useful for expression of fusion proteins. Purification of hybrid proteins containing the C-LYTA fragment at the amino terminus has been described (*see Biotechnology* 10:795-798, 1992). Within a preferred embodiment, a repeat portion of LYTA may be incorporated into a fusion protein. A repeat portion is found in the C-
20 terminal region starting at residue 178. A particularly preferred repeat portion incorporates residues 188-305.

In general, polypeptides (including fusion proteins) and polynucleotides as described herein are isolated. An "isolated" polypeptide or polynucleotide is one that is removed from its original environment. For example, a naturally-occurring protein is
25 isolated if it is separated from some or all of the coexisting materials in the natural system. Preferably, such polypeptides are at least about 90% pure, more preferably at least about 95% pure and most preferably at least about 99% pure. A polynucleotide is considered to be isolated if, for example, it is cloned into a vector that is not a part of the natural environment.

BINDING AGENTS

The present invention further provides agents, such as antibodies and antigen-binding fragments thereof, that specifically bind to a breast tumor protein. As used herein, an antibody, or antigen-binding fragment thereof, is said to "specifically bind" to a breast tumor protein if it reacts at a detectable level (within, for example, an ELISA) with a breast tumor protein, and does not react detectably with unrelated proteins under similar conditions. As used herein, "binding" refers to a noncovalent association between two separate molecules such that a complex is formed. The ability to bind may be evaluated by, for example, determining a binding constant for the formation of the complex. The binding constant is the value obtained when the concentration of the complex is divided by the product of the component concentrations. In general, two compounds are said to "bind," in the context of the present invention, when the binding constant for complex formation exceeds about 10^3 L/mol. The binding constant may be determined using methods well known in the art.

Binding agents may be further capable of differentiating between patients with and without a cancer, such as breast cancer, using the representative assays provided herein. In other words, antibodies or other binding agents that bind to a breast tumor protein will generate a signal indicating the presence of a cancer in at least about 20% of patients with the disease, and will generate a negative signal indicating the absence of the disease in at least about 90% of individuals without the cancer. To determine whether a binding agent satisfies this requirement, biological samples (*e.g.*, blood, sera, sputum, urine and/or tumor biopsies) from patients with and without a cancer (as determined using standard clinical tests) may be assayed as described herein for the presence of polypeptides that bind to the binding agent. It will be apparent that a statistically significant number of samples with and without the disease should be assayed. Each binding agent should satisfy the above criteria; however, those of ordinary skill in the art will recognize that binding agents may be used in combination to improve sensitivity.

Any agent that satisfies the above requirements may be a binding agent. For example, a binding agent may be a ribosome, with or without a peptide component, an RNA molecule or a polypeptide. In a preferred embodiment, a binding agent is an

antibody or an antigen-binding fragment thereof. Antibodies may be prepared by any of a variety of techniques known to those of ordinary skill in the art. *See, e.g.*, Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988. In general, antibodies can be produced by cell culture techniques, including the generation
5 of monoclonal antibodies as described herein, or via transfection of antibody genes into suitable bacterial or mammalian cell hosts, in order to allow for the production of recombinant antibodies. In one technique, an immunogen comprising the polypeptide
10 is initially injected into any of a wide variety of mammals (*e.g.*, mice, rats, rabbits, sheep or goats). In this step, the polypeptides of this invention may serve as the immunogen without modification. Alternatively, particularly for relatively short polypeptides, a superior immune response may be elicited if the polypeptide is joined to a carrier protein, such as bovine serum albumin or keyhole limpet hemocyanin. The immunogen is injected into the animal host, preferably according to a predetermined schedule incorporating one or more booster immunizations, and the animals are bled
15 periodically. Polyclonal antibodies specific for the polypeptide may then be purified from such antisera by, for example, affinity chromatography using the polypeptide coupled to a suitable solid support.

Monoclonal antibodies specific for an antigenic polypeptide of interest may be prepared, for example, using the technique of Kohler and Milstein, *Eur. J.*
20 *Immunol.* 6:511-519, 1976, and improvements thereto. Briefly, these methods involve the preparation of immortal cell lines capable of producing antibodies having the desired specificity (*i.e.*, reactivity with the polypeptide of interest). Such cell lines may be produced, for example, from spleen cells obtained from an animal immunized as described above. The spleen cells are then immortalized by, for example, fusion with a
25 myeloma cell fusion partner, preferably one that is syngeneic with the immunized animal. A variety of fusion techniques may be employed. For example, the spleen cells and myeloma cells may be combined with a nonionic detergent for a few minutes and then plated at low density on a selective medium that supports the growth of hybrid cells, but not myeloma cells. A preferred selection technique uses HAT (hypoxanthine,
30 aminopterin, thymidine) selection. After a sufficient time, usually about 1 to 2 weeks, colonies of hybrids are observed. Single colonies are selected and their culture

supernatants tested for binding activity against the polypeptide. Hybridomas having high reactivity and specificity are preferred.

Monoclonal antibodies may be isolated from the supernatants of growing hybridoma colonies. In addition, various techniques may be employed to enhance the yield, such as injection of the hybridoma cell line into the peritoneal cavity of a suitable vertebrate host, such as a mouse. Monoclonal antibodies may then be harvested from the ascites fluid or the blood. Contaminants may be removed from the antibodies by conventional techniques, such as chromatography, gel filtration, precipitation, and extraction. The polypeptides of this invention may be used in the purification process in, for example, an affinity chromatography step.

Within certain embodiments, the use of antigen-binding fragments of antibodies may be preferred. Such fragments include Fab fragments, which may be prepared using standard techniques. Briefly, immunoglobulins may be purified from rabbit serum by affinity chromatography on Protein A bead columns (Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory, 1988) and digested by papain to yield Fab and Fc fragments. The Fab and Fc fragments may be separated by affinity chromatography on protein A bead columns.

Monoclonal antibodies of the present invention may be coupled to one or more therapeutic agents. Suitable agents in this regard include radionuclides, differentiation inducers, drugs, toxins, and derivatives thereof. Preferred radionuclides include ^{90}Y , ^{123}I , ^{125}I , ^{131}I , ^{186}Re , ^{188}Re , ^{211}At , and ^{212}Bi . Preferred drugs include methotrexate, and pyrimidine and purine analogs. Preferred differentiation inducers include phorbol esters and butyric acid. Preferred toxins include ricin, abrin, diphtheria toxin, cholera toxin, gelonin, *Pseudomonas* exotoxin, *Shigella* toxin, and pokeweed antiviral protein.

A therapeutic agent may be coupled (e.g., covalently bonded) to a suitable monoclonal antibody either directly or indirectly (e.g., via a linker group). A direct reaction between an agent and an antibody is possible when each possesses a substituent capable of reacting with the other. For example, a nucleophilic group, such as an amino or sulfhydryl group, on one may be capable of reacting with a carbonyl-

containing group, such as an anhydride or an acid halide, or with an alkyl group containing a good leaving group (*e.g.*, a halide) on the other.

Alternatively, it may be desirable to couple a therapeutic agent and an antibody via a linker group. A linker group can function as a spacer to distance an antibody from an agent in order to avoid interference with binding capabilities. A linker group can also serve to increase the chemical reactivity of a substituent on an agent or an antibody, and thus increase the coupling efficiency. An increase in chemical reactivity may also facilitate the use of agents, or functional groups on agents, which otherwise would not be possible.

It will be evident to those skilled in the art that a variety of bifunctional or polyfunctional reagents, both homo- and hetero-functional (such as those described in the catalog of the Pierce Chemical Co., Rockford, IL), may be employed as the linker group. Coupling may be effected, for example, through amino groups, carboxyl groups, sulfhydryl groups or oxidized carbohydrate residues. There are numerous references describing such methodology, *e.g.*, U.S. Patent No. 4,671,958, to Rodwell *et al.*

Where a therapeutic agent is more potent when free from the antibody portion of the immunoconjugates of the present invention, it may be desirable to use a linker group which is cleavable during or upon internalization into a cell. A number of different cleavable linker groups have been described. The mechanisms for the intracellular release of an agent from these linker groups include cleavage by reduction of a disulfide bond (*e.g.*, U.S. Patent No. 4,489,710, to Spitler), by irradiation of a photolabile bond (*e.g.*, U.S. Patent No. 4,625,014, to Senter *et al.*), by hydrolysis of derivatized amino acid side chains (*e.g.*, U.S. Patent No. 4,638,045, to Kohn *et al.*), by serum complement-mediated hydrolysis (*e.g.*, U.S. Patent No. 4,671,958, to Rodwell *et al.*), and acid-catalyzed hydrolysis (*e.g.*, U.S. Patent No. 4,569,789, to Blattler *et al.*).

It may be desirable to couple more than one agent to an antibody. In one embodiment, multiple molecules of an agent are coupled to one antibody molecule. In another embodiment, more than one type of agent may be coupled to one antibody. Regardless of the particular embodiment, immunoconjugates with more than one agent may be prepared in a variety of ways. For example, more than one agent may be

coupled directly to an antibody molecule, or linkers that provide multiple sites for attachment can be used. Alternatively, a carrier can be used.

A carrier may bear the agents in a variety of ways, including covalent bonding either directly or via a linker group. Suitable carriers include proteins such as albumins (*e.g.*, U.S. Patent No. 4,507,234, to Kato *et al.*), peptides and polysaccharides such as aminodextran (*e.g.*, U.S. Patent No. 4,699,784, to Shih *et al.*). A carrier may also bear an agent by noncovalent bonding or by encapsulation, such as within a liposome vesicle (*e.g.*, U.S. Patent Nos. 4,429,008 and 4,873,088). Carriers specific for radionuclide agents include radiohalogenated small molecules and chelating compounds. For example, U.S. Patent No. 4,735,792 discloses representative radiohalogenated small molecules and their synthesis. A radionuclide chelate may be formed from chelating compounds that include those containing nitrogen and sulfur atoms as the donor atoms for binding the metal, or metal oxide, radionuclide. For example, U.S. Patent No. 4,673,562, to Davison *et al.* discloses representative chelating compounds and their synthesis.

A variety of routes of administration for the antibodies and immunoconjugates may be used. Typically, administration will be intravenous, intramuscular, subcutaneous or in the bed of a resected tumor. It will be evident that the precise dose of the antibody/immunoconjugate will vary depending upon the antibody used, the antigen density on the tumor, and the rate of clearance of the antibody.

T CELLS

Immunotherapeutic compositions may also, or alternatively, comprise T cells specific for a breast tumor protein. Such cells may generally be prepared *in vitro* or *ex vivo*, using standard procedures. For example, T cells may be isolated from bone marrow, peripheral blood, or a fraction of bone marrow or peripheral blood of a patient, using a commercially available cell separation system, such as the Isolex™ System, available from Nexell Therapeutics, Inc. (Irvine, CA; see also U.S. Patent No. 5,240,856; U.S. Patent No. 5,215,926; WO 89/06280; WO 91/16116 and WO

92/07243). Alternatively, T cells may be derived from related or unrelated humans, non-human mammals, cell lines or cultures.

T cells may be stimulated with a breast tumor polypeptide, polynucleotide encoding a breast tumor polypeptide and/or an antigen presenting cell (APC) that expresses such a polypeptide. Such stimulation is performed under conditions and for a time sufficient to permit the generation of T cells that are specific for the polypeptide. Preferably, a breast tumor polypeptide or polynucleotide is present within a delivery vehicle, such as a microsphere, to facilitate the generation of specific T cells.

T cells are considered to be specific for a breast tumor polypeptide if the T cells specifically proliferate, secrete cytokines or kill target cells coated with the polypeptide or expressing a gene encoding the polypeptide. T cell specificity may be evaluated using any of a variety of standard techniques. For example, within a chromium release assay or proliferation assay, a stimulation index of more than two fold increase in lysis and/or proliferation, compared to negative controls, indicates T cell specificity. Such assays may be performed, for example, as described in Chen *et al.*, *Cancer Res.* 54:1065-1070, 1994. Alternatively, detection of the proliferation of T cells may be accomplished by a variety of known techniques. For example, T cell proliferation can be detected by measuring an increased rate of DNA synthesis (*e.g.*, by pulse-labeling cultures of T cells with tritiated thymidine and measuring the amount of tritiated thymidine incorporated into DNA). Contact with a breast tumor polypeptide (100 ng/ml - 100 µg/ml, preferably 200 ng/ml - 25 µg/ml) for 3 - 7 days should result in at least a two fold increase in proliferation of the T cells. Contact as described above for 2-3 hours should result in activation of the T cells, as measured using standard cytokine assays in which a two fold increase in the level of cytokine release (*e.g.*, TNF or IFN-γ) is indicative of T cell activation (*see* Coligan *et al.*, *Current Protocols in Immunology*, vol. 1, Wiley Interscience (Greene 1998)). T cells that have been activated in response to a breast tumor polypeptide, polynucleotide or polypeptide-expressing APC may be CD4⁺ and/or CD8⁺. Breast tumor protein-specific T cells may be expanded using standard techniques. Within preferred embodiments, the T cells are

derived from a patient, a related donor or an unrelated donor, and are administered to the patient following stimulation and expansion.

For therapeutic purposes, CD4⁺ or CD8⁺ T cells that proliferate in response to a breast tumor polypeptide, polynucleotide or APC can be expanded in number either *in vitro* or *in vivo*. Proliferation of such T cells *in vitro* may be accomplished in a variety of ways. For example, the T cells can be re-exposed to a breast tumor polypeptide, or a short peptide corresponding to an immunogenic portion of such a polypeptide, with or without the addition of T cell growth factors, such as interleukin-2, and/or stimulator cells that synthesize a breast tumor polypeptide. Alternatively, one or more T cells that proliferate in the presence of a breast tumor protein can be expanded in number by cloning. Methods for cloning cells are well known in the art, and include limiting dilution.

PHARMACEUTICAL COMPOSITIONS

In additional embodiments, the present invention concerns formulation of one or more of the polynucleotide, polypeptide, T-cell and/or antibody compositions disclosed herein in pharmaceutically-acceptable solutions for administration to a cell or an animal, either alone, or in combination with one or more other modalities of therapy.

It will also be understood that, if desired, the nucleic acid segment, RNA, DNA or PNA compositions that express a polypeptide as disclosed herein may be administered in combination with other agents as well, such as, *e.g.*, other proteins or polypeptides or various pharmaceutically-active agents. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The compositions may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or alternatively may be chemically synthesized as described herein. Likewise, such compositions may further comprise substituted or derivatized RNA or DNA compositions.

Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable

dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.*, oral, parenteral, intravenous, intranasal, and intramuscular administration and formulation.

1. ORAL DELIVERY

5 In certain applications, the pharmaceutical compositions disclosed herein may be delivered *via* oral administration to an animal. As such, these compositions may be formulated with an inert diluent or with an assimilable edible carrier, or they may be enclosed in hard- or soft-shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet.

10 The active compounds may even be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like (Mathiowitz *et al.*, 1997; Hwang *et al.*, 1998; U. S. Patent 5,641,515; U. S. Patent 5,580,579 and U. S. Patent 5,792,451, each specifically incorporated herein by reference in its entirety). The tablets, troches, pills,
15 capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry
20 flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar, or both. A syrup of elixir may contain the active compound sucrose as a sweetening agent methyl and
25 propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compounds may be incorporated into sustained-release preparation and formulations.

Typically, these formulations may contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 60% or 70% or more of the weight or volume of the total formulation. Naturally, the amount of active compound(s) in each therapeutically useful composition may be prepared in such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

For oral administration the compositions of the present invention may alternatively be incorporated with one or more excipients in the form of a mouthwash, dentifrice, buccal tablet, oral spray, or sublingual orally-administered formulation. For example, a mouthwash may be prepared incorporating the active ingredient in the required amount in an appropriate solvent, such as a sodium borate solution (Dobell's Solution). Alternatively, the active ingredient may be incorporated into an oral solution such as one containing sodium borate, glycerin and potassium bicarbonate, or dispersed in a dentifrice, or added in a therapeutically-effective amount to a composition that may include water, binders, abrasives, flavoring agents, foaming agents, and humectants. Alternatively the compositions may be fashioned into a tablet or solution form that may be placed under the tongue or otherwise dissolved in the mouth.

2. INJECTABLE DELIVERY

In certain circumstances it will be desirable to deliver the pharmaceutical compositions disclosed herein parenterally, intravenously, intramuscularly, or even intraperitoneally as described in U. S. Patent 5,543,158; U. S. Patent 5,641,515 and U. S. Patent 5,399,363 (each specifically incorporated herein by reference in its entirety). Solutions of the active compounds as free base or pharmacologically acceptable salts may be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of

storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U. S. Patent 5,466,468, specifically incorporated herein by reference in its entirety). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be facilitated by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event,

determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

5 Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the
10 preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The compositions disclosed herein may be formulated in a neutral or salt
15 form. Pharmaceutically-acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium,
20 ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

25 As used herein, "carrier" includes any and all solvents, dispersion media, vehicles, coatings, diluents, antibacterial and antifungal agents, isotonic and absorption delaying agents, buffers, carrier solutions, suspensions, colloids, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active
30 ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

The phrase "pharmaceutically-acceptable" refers to molecular entities and compositions that do not produce an allergic or similar untoward reaction when administered to a human. The preparation of an aqueous composition that contains a protein as an active ingredient is well understood in the art. Typically, such compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection can also be prepared. The preparation can also be emulsified.

3. NASAL DELIVERY

In certain embodiments, the pharmaceutical compositions may be delivered by intranasal sprays, inhalation, and/or other aerosol delivery vehicles. Methods for delivering genes, nucleic acids, and peptide compositions directly to the lungs *via* nasal aerosol sprays has been described *e.g.*, in U. S. Patent 5,756,353 and U. S. Patent 5,804,212 (each specifically incorporated herein by reference in its entirety). Likewise, the delivery of drugs using intranasal microparticle resins (Takenaga *et al.*, 1998) and lysophosphatidyl-glycerol compounds (U. S. Patent 5,725,871, specifically incorporated herein by reference in its entirety) are also well-known in the pharmaceutical arts. Likewise, transmucosal drug delivery in the form of a polytetrafluoroethylene support matrix is described in U. S. Patent 5,780,045 (specifically incorporated herein by reference in its entirety).

4. LIPOSOME-, NANOCAPSULE-, AND MICROPARTICLE-MEDIATED DELIVERY

In certain embodiments, the inventors contemplate the use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present invention into suitable host cells. In particular, the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

Such formulations may be preferred for the introduction of pharmaceutically-acceptable formulations of the nucleic acids or constructs disclosed herein. The formation and use of liposomes is generally known to those of skill in the

art (see for example, Couvreur *et al.*, 1977; Couvreur, 1988; Lasic, 1998; which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy for intracellular bacterial infections and diseases). Recently, liposomes were developed with improved serum stability and circulation half-times (Gabizon and Papahadjopoulos, 1988; Allen and Choun, 1987; U. S. Patent 5,741,516, specifically incorporated herein by reference in its entirety). Further, various methods of liposome and liposome like preparations as potential drug carriers have been reviewed (Takakura, 1998; Chandran *et al.*, 1997; Margalit, 1995; U. S. Patent 5,567,434; U. S. Patent 5,552,157; U. S. Patent 5,565,213; U. S. Patent 5,738,868 and U. S. Patent 5,795,587, each specifically incorporated herein by reference in its entirety).

Liposomes have been used successfully with a number of cell types that are normally resistant to transfection by other procedures including T cell suspensions, primary hepatocyte cultures and PC 12 cells (Renneisen *et al.*, 1990; Muller *et al.*, 1990). In addition, liposomes are free of the DNA length constraints that are typical of viral-based delivery systems. Liposomes have been used effectively to introduce genes, drugs (Heath and Martin, 1986; Heath *et al.*, 1986; Balazsovits *et al.*, 1989; Fresta and Puglisi, 1996), radiotherapeutic agents (Pikul *et al.*, 1987), enzymes (Imaizumi *et al.*, 1990a; Imaizumi *et al.*, 1990b), viruses (Faller and Baltimore, 1984), transcription factors and allosteric effectors (Nicolau and Gersonde, 1979) into a variety of cultured cell lines and animals. In addition, several successful clinical trials examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein *et al.*, 1985a; 1985b; Coune, 1988; Sculier *et al.*, 1988). Furthermore, several studies suggest that the use of liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mori and Fukatsu, 1992).

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 μ m. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

Liposomes bear resemblance to cellular membranes and are contemplated for use in connection with the present invention as carriers for the peptide compositions. They are widely suitable as both water- and lipid-soluble substances can be entrapped, *i.e.* in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

In addition to the teachings of Couvreur *et al.* (1977; 1988), the following information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins, such as cytochrome c, bind, deform and penetrate the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution, however, and a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUVs). These are prepared by ether evaporation and are three to four times more efficient at solute entrapment than MLVs.

In addition to liposome characteristics, an important determinant in entrapping compounds is the physicochemical properties of the compound itself. Polar compounds are trapped in the aqueous spaces and nonpolar compounds bind to the lipid bilayer of the vesicle. Polar compounds are released through permeation or when the bilayer is broken, but nonpolar compounds remain affiliated with the bilayer unless it is disrupted by temperature or exposure to lipoproteins. Both types show maximum efflux rates at the phase transition temperature.

Liposomes interact with cells *via* four different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. It often is difficult to determine which mechanism is operative and more than one may operate at the same time.

The fate and disposition of intravenously injected liposomes depend on their physical properties, such as size, fluidity, and surface charge. They may persist in tissues for h or days, depending on their composition, and half lives in the blood range from min to several h. Larger liposomes, such as MLVs and LUVs, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but physiology of the circulatory system restrains the exit of such large species at most sites. They can exit only in places where large openings or pores exist in the capillary endothelium, such as the sinusoids of the liver or spleen. Thus, these organs are the predominate site of uptake. On the other hand, SUVs show a broader tissue distribution but still are sequestered highly in the liver and spleen. In general, this *in vivo* behavior limits the potential targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow, and lymphoid organs.

Targeting is generally not a limitation in terms of the present invention. However, should specific targeting be desired, methods are available for this to be accomplished. Antibodies may be used to bind to the liposome surface and to direct the

antibody and its drug contents to specific antigenic receptors located on a particular cell-type surface. Carbohydrate determinants (glycoprotein or glycolipid cell-surface components that play a role in cell-cell recognition, interaction and adhesion) may also be used as recognition sites as they have potential in directing liposomes to particular cell types. Mostly, it is contemplated that intravenous injection of liposomal preparations would be used, but other routes of administration are also conceivable.

Alternatively, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland *et al.*, 1987; Quintanar-Guerrero *et al.*, 1998; Douglas *et al.*, 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μm) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention. Such particles may be easily made, as described (Couvreur *et al.*, 1980; 1988; zur Muhlen *et al.*, 1998; Zambaux *et al.* 1998; Pinto-Alphandry *et al.*, 1995 and U. S. Patent 5,145,684, specifically incorporated herein by reference in its entirety).

VACCINES

In certain preferred embodiments of the present invention, vaccines are provided. The vaccines will generally comprise one or more pharmaceutical compositions, such as those discussed above, in combination with an immunostimulant. An immunostimulant may be any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen. Examples of immunostimulants include adjuvants, biodegradable microspheres (*e.g.*, polylactic galactide) and liposomes (into which the compound is incorporated; *see e.g.*, Fullerton, U.S. Patent No. 4,235,877). Vaccine preparation is generally described in, for example, M.F. Powell and M.J. Newman, eds., "Vaccine Design (the subunit and adjuvant approach)," Plenum Press (NY, 1995). Pharmaceutical compositions and vaccines within the scope of the present invention may also contain other compounds, which may be biologically active or inactive. For example, one or more immunogenic

portions of other tumor antigens may be present, either incorporated into a fusion polypeptide or as a separate compound, within the composition or vaccine.

Illustrative vaccines may contain DNA encoding one or more of the polypeptides as described above, such that the polypeptide is generated *in situ*. As noted above, the DNA may be present within any of a variety of delivery systems known to those of ordinary skill in the art, including nucleic acid expression systems, bacteria and viral expression systems. Numerous gene delivery techniques are well known in the art, such as those described by Rolland, *Crit. Rev. Therap. Drug Carrier Systems* 15:143-198, 1998, and references cited therein. Appropriate nucleic acid expression systems contain the necessary DNA sequences for expression in the patient (such as a suitable promoter and terminating signal). Bacterial delivery systems involve the administration of a bacterium (such as *Bacillus-Calmette-Guerrin*) that expresses an immunogenic portion of the polypeptide on its cell surface or secretes such an epitope. In a preferred embodiment, the DNA may be introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus. Suitable systems are disclosed, for example, in Fisher-Hoch *et al.*, *Proc. Natl. Acad. Sci. USA* 86:317-321, 1989; Flexner *et al.*, *Ann. N.Y. Acad. Sci.* 569:86-103, 1989; Flexner *et al.*, *Vaccine* 8:17-21, 1990; U.S. Patent Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973; U.S. Patent No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner, *Biotechniques* 6:616-627, 1988; Rosenfeld *et al.*, *Science* 252:431-434, 1991; Kolls *et al.*, *Proc. Natl. Acad. Sci. USA* 91:215-219, 1994; Kass-Eisler *et al.*, *Proc. Natl. Acad. Sci. USA* 90:11498-11502, 1993; Guzman *et al.*, *Circulation* 88:2838-2848, 1993; and Guzman *et al.*, *Cir. Res.* 73:1202-1207, 1993. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer *et al.*, *Science* 259:1745-1749, 1993 and reviewed by Cohen, *Science* 259:1691-1692, 1993. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads, which are efficiently transported into the cells. It will be apparent that a vaccine may comprise both a polynucleotide and a polypeptide component. Such vaccines may provide for an enhanced immune response.

It will be apparent that a vaccine may contain pharmaceutically acceptable salts of the polynucleotides and polypeptides provided herein. Such salts may be prepared from pharmaceutically acceptable non-toxic bases, including organic bases (*e.g.*, salts of primary, secondary and tertiary amines and basic amino acids) and
5 inorganic bases (*e.g.*, sodium, potassium, lithium, ammonium, calcium and magnesium salts).

While any suitable carrier known to those of ordinary skill in the art may be employed in the vaccine compositions of this invention, the type of carrier will vary depending on the mode of administration. Compositions of the present invention may
10 be formulated for any appropriate manner of administration, including for example, topical, oral, nasal, intravenous, intracranial, intraperitoneal, subcutaneous or intramuscular administration. For parenteral administration, such as subcutaneous injection, the carrier preferably comprises water, saline, alcohol, a fat, a wax or a buffer. For oral administration, any of the above carriers or a solid carrier, such as mannitol,
15 lactose, starch, magnesium stearate, sodium saccharine, talcum, cellulose, glucose, sucrose, and magnesium carbonate, may be employed. Biodegradable microspheres (*e.g.*, polylactate polyglycolate) may also be employed as carriers for the pharmaceutical compositions of this invention. Suitable biodegradable microspheres are disclosed, for example, in U.S. Patent Nos. 4,897,268; 5,075,109; 5,928,647;
20 5,811,128; 5,820,883; 5,853,763; 5,814,344 and 5,942,252. One may also employ a carrier comprising the particulate-protein complexes described in U.S. Patent No. 5,928,647, which are capable of inducing a class I-restricted cytotoxic T lymphocyte responses in a host.

Such compositions may also comprise buffers (*e.g.*, neutral buffered
25 saline or phosphate buffered saline), carbohydrates (*e.g.*, glucose, mannose, sucrose or dextrans), mannitol, proteins, polypeptides or amino acids such as glycine, antioxidants, bacteriostats, chelating agents such as EDTA or glutathione, adjuvants (*e.g.*, aluminum hydroxide), solutes that render the formulation isotonic, hypotonic or weakly hypertonic with the blood of a recipient, suspending agents, thickening agents and/or preservatives.
30 Alternatively, compositions of the present invention may be formulated as a

lyophilizate. Compounds may also be encapsulated within liposomes using well known technology.

Any of a variety of immunostimulants may be employed in the vaccines of this invention. For example, an adjuvant may be included. Most adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *Mycobacterium tuberculosis* derived proteins. Suitable adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres; monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF or interleukin-2, -7, or -12, may also be used as adjuvants.

Within the vaccines provided herein, the adjuvant composition is preferably designed to induce an immune response predominantly of the Th1 type. High levels of Th1-type cytokines (e.g., IFN- γ , TNF α , IL-2 and IL-12) tend to favor the induction of cell mediated immune responses to an administered antigen. In contrast, high levels of Th2-type cytokines (e.g., IL-4, IL-5, IL-6 and IL-10) tend to favor the induction of humoral immune responses. Following application of a vaccine as provided herein, a patient will support an immune response that includes Th1- and Th2-type responses. Within a preferred embodiment, in which a response is predominantly Th1-type, the level of Th1-type cytokines will increase to a greater extent than the level of Th2-type cytokines. The levels of these cytokines may be readily assessed using standard assays. For a review of the families of cytokines, see Mosmann and Coffman, *Ann. Rev. Immunol.* 7:145-173, 1989.

Preferred adjuvants for use in eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A (3D-MPL), together with an aluminum salt. MPL adjuvants are available from Corixa Corporation (Seattle, WA; see US Patent

Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato *et al.*, *Science* 273:352, 1996. Another preferred adjuvant is a saponin, preferably QS21 (Aquila Biopharmaceuticals Inc., Framingham, MA), which may be used alone or in combination with other adjuvants. For example, an enhanced system involves the combination of a monophosphoryl lipid A and saponin derivative, such as the combination of QS21 and 3D-MPL as described in WO 94/00153, or a less reactogenic composition where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other preferred formulations comprise an oil-in-water emulsion and tocopherol. A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

Other preferred adjuvants include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties.

Any vaccine provided herein may be prepared using well known methods that result in a combination of antigen, immune response enhancer and a suitable carrier or excipient. The compositions described herein may be administered as part of a sustained release formulation (*i.e.*, a formulation such as a capsule, sponge or gel (composed of polysaccharides, for example) that effects a slow release of compound following administration). Such formulations may generally be prepared using well known technology (*see, e.g.*, Coombes *et al.*, *Vaccine* 14:1429-1438, 1996) and administered by, for example, oral, rectal or subcutaneous implantation, or by implantation at the desired target site. Sustained-release formulations may contain a

polypeptide, polynucleotide or antibody dispersed in a carrier matrix and/or contained within a reservoir surrounded by a rate controlling membrane.

Carriers for use within such formulations are biocompatible, and may also be biodegradable; preferably the formulation provides a relatively constant level of active component release. Such carriers include microparticles of poly(lactide-co-glycolide), polyacrylate, latex, starch, cellulose, dextran and the like. Other delayed-release carriers include supramolecular biovectors, which comprise a non-liquid hydrophilic core (*e.g.*, a cross-linked polysaccharide or oligosaccharide) and, optionally, an external layer comprising an amphiphilic compound, such as a phospholipid (*see e.g.*, U.S. Patent No. 5,151,254 and PCT applications WO 94/20078, WO/94/23701 and WO 96/06638). The amount of active compound contained within a sustained release formulation depends upon the site of implantation, the rate and expected duration of release and the nature of the condition to be treated or prevented.

Any of a variety of delivery vehicles may be employed within pharmaceutical compositions and vaccines to facilitate production of an antigen-specific immune response that targets tumor cells. Delivery vehicles include antigen presenting cells (APCs), such as dendritic cells, macrophages, B cells, monocytes and other cells that may be engineered to be efficient APCs. Such cells may, but need not, be genetically modified to increase the capacity for presenting the antigen, to improve activation and/or maintenance of the T cell response, to have anti-tumor effects *per se* and/or to be immunologically compatible with the receiver (*i.e.*, matched HLA haplotype). APCs may generally be isolated from any of a variety of biological fluids and organs, including tumor and peritumoral tissues, and may be autologous, allogeneic, syngeneic or xenogeneic cells.

Certain preferred embodiments of the present invention use dendritic cells or progenitors thereof as antigen-presenting cells. Dendritic cells are highly potent APCs (Banchereau and Steinman, *Nature* 392:245-251, 1998) and have been shown to be effective as a physiological adjuvant for eliciting prophylactic or therapeutic antitumor immunity (*see* Timmerman and Levy, *Ann. Rev. Med.* 50:507-529, 1999). In general, dendritic cells may be identified based on their typical shape (stellate *in situ*, with marked cytoplasmic processes (dendrites) visible *in vitro*), their ability to take up,

process and present antigens with high efficiency and their ability to activate naïve T cell responses. Dendritic cells may, of course, be engineered to express specific cell-surface receptors or ligands that are not commonly found on dendritic cells *in vivo* or *ex vivo*, and such modified dendritic cells are contemplated by the present invention. As
5 an alternative to dendritic cells, secreted vesicles antigen-loaded dendritic cells (called exosomes) may be used within a vaccine (*see Zitvogel et al., Nature Med. 4:594-600, 1998*).

Dendritic cells and progenitors may be obtained from peripheral blood, bone marrow, tumor-infiltrating cells, peritumoral tissues-infiltrating cells, lymph
10 nodes, spleen, skin, umbilical cord blood or any other suitable tissue or fluid. For example, dendritic cells may be differentiated *ex vivo* by adding a combination of cytokines such as GM-CSF, IL-4, IL-13 and/or TNF α to cultures of monocytes harvested from peripheral blood. Alternatively, CD34 positive cells harvested from peripheral blood, umbilical cord blood or bone marrow may be differentiated into
15 dendritic cells by adding to the culture medium combinations of GM-CSF, IL-3, TNF α , CD40 ligand, LPS, flt3 ligand and/or other compound(s) that induce differentiation, maturation and proliferation of dendritic cells.

Dendritic cells are conveniently categorized as "immature" and "mature" cells, which allows a simple way to discriminate between two well characterized
20 phenotypes. However, this nomenclature should not be construed to exclude all possible intermediate stages of differentiation. Immature dendritic cells are characterized as APC with a high capacity for antigen uptake and processing, which correlates with the high expression of Fc γ receptor and mannose receptor. The mature phenotype is typically characterized by a lower expression of these markers, but a high
25 expression of cell surface molecules responsible for T cell activation such as class I and class II MHC, adhesion molecules (*e.g.*, CD54 and CD11) and costimulatory molecules (*e.g.*, CD40, CD80, CD86 and 4-1BB).

APCs may generally be transfected with a polynucleotide encoding a breast tumor protein (or portion or other variant thereof) such that the breast tumor
30 polypeptide, or an immunogenic portion thereof, is expressed on the cell surface. Such transfection may take place *ex vivo*, and a composition or vaccine comprising such

transfected cells may then be used for therapeutic purposes, as described herein. Alternatively, a gene delivery vehicle that targets a dendritic or other antigen presenting cell may be administered to a patient, resulting in transfection that occurs *in vivo*. *In vivo* and *ex vivo* transfection of dendritic cells, for example, may generally be performed using any methods known in the art, such as those described in WO 97/24447, or the gene gun approach described by Mahvi *et al.*, *Immunology and cell Biology* 75:456-460, 1997. Antigen loading of dendritic cells may be achieved by incubating dendritic cells or progenitor cells with the breast tumor polypeptide, DNA (naked or within a plasmid vector) or RNA; or with antigen-expressing recombinant bacterium or viruses (e.g., vaccinia, fowlpox, adenovirus or lentivirus vectors). Prior to loading, the polypeptide may be covalently conjugated to an immunological partner that provides T cell help (e.g., a carrier molecule). Alternatively, a dendritic cell may be pulsed with a non-conjugated immunological partner, separately or in the presence of the polypeptide.

Vaccines and pharmaceutical compositions may be presented in unit-dose or multi-dose containers, such as sealed ampoules or vials. Such containers are preferably hermetically sealed to preserve sterility of the formulation until use. In general, formulations may be stored as suspensions, solutions or emulsions in oily or aqueous vehicles. Alternatively, a vaccine or pharmaceutical composition may be stored in a freeze-dried condition requiring only the addition of a sterile liquid carrier immediately prior to use.

CANCER THERAPY

In further aspects of the present invention, the compositions described herein may be used for immunotherapy of cancer, such as breast cancer. Within such methods, pharmaceutical compositions and vaccines are typically administered to a patient. As used herein, a "patient" refers to any warm-blooded animal, preferably a human. A patient may or may not be afflicted with cancer. Accordingly, the above pharmaceutical compositions and vaccines may be used to prevent the development of a cancer or to treat a patient afflicted with a cancer. A cancer may be diagnosed using criteria generally accepted in the art, including the presence of a malignant tumor.

Pharmaceutical compositions and vaccines may be administered either prior to or following surgical removal of primary tumors and/or treatment such as administration of radiotherapy or conventional chemotherapeutic drugs. Administration may be by any suitable method, including administration by intravenous, intraperitoneal, 5 intramuscular, subcutaneous, intranasal, intradermal, anal, vaginal, topical and oral routes.

Within certain embodiments, immunotherapy may be active immunotherapy, in which treatment relies on the *in vivo* stimulation of the endogenous host immune system to react against tumors with the administration of immune 10 response-modifying agents (such as polypeptides and polynucleotides as provided herein).

Within other embodiments, immunotherapy may be passive immunotherapy, in which treatment involves the delivery of agents with established tumor-immune reactivity (such as effector cells or antibodies) that can directly or 15 indirectly mediate antitumor effects and does not necessarily depend on an intact host immune system. Examples of effector cells include T cells as discussed above, T lymphocytes (such as CD8⁺ cytotoxic T lymphocytes and CD4⁺ T-helper tumor-infiltrating lymphocytes), killer cells (such as Natural Killer cells and lymphokine-activated killer cells), B cells and antigen-presenting cells (such as dendritic cells and 20 macrophages) expressing a polypeptide provided herein. T cell receptors and antibody receptors specific for the polypeptides recited herein may be cloned, expressed and transferred into other vectors or effector cells for adoptive immunotherapy. The polypeptides provided herein may also be used to generate antibodies or anti-idiotypic antibodies (as described above and in U.S. Patent No. 4,918,164) for passive 25 immunotherapy.

Effector cells may generally be obtained in sufficient quantities for adoptive immunotherapy by growth *in vitro*, as described herein. Culture conditions for expanding single antigen-specific effector cells to several billion in number with retention of antigen recognition *in vivo* are well known in the art. Such *in vitro* culture 30 conditions typically use intermittent stimulation with antigen, often in the presence of cytokines (such as IL-2) and non-dividing feeder cells. As noted above,

immunoreactive polypeptides as provided herein may be used to rapidly expand antigen-specific T cell cultures in order to generate a sufficient number of cells for immunotherapy. In particular, antigen-presenting cells, such as dendritic, macrophage, monocyte, fibroblast and/or B cells, may be pulsed with immunoreactive polypeptides or transfected with one or more polynucleotides using standard techniques well known in the art. For example, antigen-presenting cells can be transfected with a polynucleotide having a promoter appropriate for increasing expression in a recombinant virus or other expression system. Cultured effector cells for use in therapy must be able to grow and distribute widely, and to survive long term *in vivo*. Studies have shown that cultured effector cells can be induced to grow in vivo and to survive long term in substantial numbers by repeated stimulation with antigen supplemented with IL-2 (*see, for example, Cheever et al., Immunological Reviews 157:177, 1997*).

Alternatively, a vector expressing a polypeptide recited herein may be introduced into antigen presenting cells taken from a patient and clonally propagated *ex vivo* for transplant back into the same patient. Transfected cells may be reintroduced into the patient using any means known in the art, preferably in sterile form by intravenous, intracavitary, intraperitoneal or intratumor administration.

Routes and frequency of administration of the therapeutic compositions described herein, as well as dosage, will vary from individual to individual, and may be readily established using standard techniques. In general, the pharmaceutical compositions and vaccines may be administered by injection (*e.g.*, intracutaneous, intramuscular, intravenous or subcutaneous), intranasally (*e.g.*, by aspiration) or orally. Preferably, between 1 and 10 doses may be administered over a 52 week period. Preferably, 6 doses are administered, at intervals of 1 month, and booster vaccinations may be given periodically thereafter. Alternate protocols may be appropriate for individual patients. A suitable dose is an amount of a compound that, when administered as described above, is capable of promoting an anti-tumor immune response, and is at least 10-50% above the basal (*i.e.*, untreated) level. Such response can be monitored by measuring the anti-tumor antibodies in a patient or by vaccine-dependent generation of cytolytic effector cells capable of killing the patient's tumor cells *in vitro*. Such vaccines should also be capable of causing an immune response that

leads to an improved clinical outcome (e.g., more frequent remissions, complete or partial or longer disease-free survival) in vaccinated patients as compared to non-vaccinated patients. In general, for pharmaceutical compositions and vaccines comprising one or more polypeptides, the amount of each polypeptide present in a dose
5 ranges from about 25 µg to 5 mg per kg of host. Suitable dose sizes will vary with the size of the patient, but will typically range from about 0.1 mL to about 5 mL.

In general, an appropriate dosage and treatment regimen provides the active compound(s) in an amount sufficient to provide therapeutic and/or prophylactic benefit. Such a response can be monitored by establishing an improved clinical
10 outcome (e.g., more frequent remissions, complete or partial, or longer disease-free survival) in treated patients as compared to non-treated patients. Increases in preexisting immune responses to a breast tumor protein generally correlate with an improved clinical outcome. Such immune responses may generally be evaluated using standard proliferation, cytotoxicity or cytokine assays, which may be performed using
15 samples obtained from a patient before and after treatment.

CANCER DETECTION AND DIAGNOSIS

In general, a cancer may be detected in a patient based on the presence of one or more breast tumor proteins and/or polynucleotides encoding such proteins in a biological sample (for example, blood, sera, sputum urine and/or tumor biopsies)
20 obtained from the patient. In other words, such proteins may be used as markers to indicate the presence or absence of a cancer such as breast cancer. In addition, such proteins may be useful for the detection of other cancers. The binding agents provided herein generally permit detection of the level of antigen that binds to the agent in the biological sample. Polynucleotide primers and probes may be used to detect the level
25 of mRNA encoding a tumor protein, which is also indicative of the presence or absence of a cancer. In general, a breast tumor sequence should be present at a level that is at least three fold higher in tumor tissue than in normal tissue

There are a variety of assay formats known to those of ordinary skill in the art for using a binding agent to detect polypeptide markers in a sample. *See, e.g.,*
30 Harlow and Lane, *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory,

1988. In general, the presence or absence of a cancer in a patient may be determined by (a) contacting a biological sample obtained from a patient with a binding agent; (b) detecting in the sample a level of polypeptide that binds to the binding agent; and (c) comparing the level of polypeptide with a predetermined cut-off value.

5 In a preferred embodiment, the assay involves the use of binding agent immobilized on a solid support to bind to and remove the polypeptide from the remainder of the sample. The bound polypeptide may then be detected using a detection reagent that contains a reporter group and specifically binds to the binding agent/polypeptide complex. Such detection reagents may comprise, for example, a
10 binding agent that specifically binds to the polypeptide or an antibody or other agent that specifically binds to the binding agent, such as an anti-immunoglobulin, protein G, protein A or a lectin. Alternatively, a competitive assay may be utilized, in which a polypeptide is labeled with a reporter group and allowed to bind to the immobilized binding agent after incubation of the binding agent with the sample. The extent to
15 which components of the sample inhibit the binding of the labeled polypeptide to the binding agent is indicative of the reactivity of the sample with the immobilized binding agent. Suitable polypeptides for use within such assays include full length breast tumor proteins and portions thereof to which the binding agent binds, as described above.

 The solid support may be any material known to those of ordinary skill
20 in the art to which the tumor protein may be attached. For example, the solid support may be a test well in a microtiter plate or a nitrocellulose or other suitable membrane. Alternatively, the support may be a bead or disc, such as glass, fiberglass, latex or a plastic material such as polystyrene or polyvinylchloride. The support may also be a magnetic particle or a fiber optic sensor, such as those disclosed, for example, in U.S.
25 Patent No. 5,359,681. The binding agent may be immobilized on the solid support using a variety of techniques known to those of skill in the art, which are amply described in the patent and scientific literature. In the context of the present invention, the term "immobilization" refers to both noncovalent association, such as adsorption, and covalent attachment (which may be a direct linkage between the agent and
30 functional groups on the support or may be a linkage by way of a cross-linking agent). Immobilization by adsorption to a well in a microtiter plate or to a membrane is

preferred. In such cases, adsorption may be achieved by contacting the binding agent, in a suitable buffer, with the solid support for a suitable amount of time. The contact time varies with temperature, but is typically between about 1 hour and about 1 day. In general, contacting a well of a plastic microtiter plate (such as polystyrene or polyvinylchloride) with an amount of binding agent ranging from about 10 ng to about 10 µg, and preferably about 100 ng to about 1 µg, is sufficient to immobilize an adequate amount of binding agent.

Covalent attachment of binding agent to a solid support may generally be achieved by first reacting the support with a bifunctional reagent that will react with both the support and a functional group, such as a hydroxyl or amino group, on the binding agent. For example, the binding agent may be covalently attached to supports having an appropriate polymer coating using benzoquinone or by condensation of an aldehyde group on the support with an amine and an active hydrogen on the binding partner (*see, e.g.,* Pierce Immunotechnology Catalog and Handbook, 1991, at A12-A13).

In certain embodiments, the assay is a two-antibody sandwich assay. This assay may be performed by first contacting an antibody that has been immobilized on a solid support, commonly the well of a microtiter plate, with the sample, such that polypeptides within the sample are allowed to bind to the immobilized antibody. Unbound sample is then removed from the immobilized polypeptide-antibody complexes and a detection reagent (preferably a second antibody capable of binding to a different site on the polypeptide) containing a reporter group is added. The amount of detection reagent that remains bound to the solid support is then determined using a method appropriate for the specific reporter group.

More specifically, once the antibody is immobilized on the support as described above, the remaining protein binding sites on the support are typically blocked. Any suitable blocking agent known to those of ordinary skill in the art, such as bovine serum albumin or Tween 20™ (Sigma Chemical Co., St. Louis, MO). The immobilized antibody is then incubated with the sample, and polypeptide is allowed to bind to the antibody. The sample may be diluted with a suitable diluent, such as phosphate-buffered saline (PBS) prior to incubation. In general, an appropriate contact

time (*i.e.*, incubation time) is a period of time that is sufficient to detect the presence of polypeptide within a sample obtained from an individual with breast cancer. Preferably, the contact time is sufficient to achieve a level of binding that is at least about 95% of that achieved at equilibrium between bound and unbound polypeptide.

- 5 Those of ordinary skill in the art will recognize that the time necessary to achieve equilibrium may be readily determined by assaying the level of binding that occurs over a period of time. At room temperature, an incubation time of about 30 minutes is generally sufficient.

- 10 Unbound sample may then be removed by washing the solid support with an appropriate buffer, such as PBS containing 0.1% Tween 20™. The second antibody, which contains a reporter group, may then be added to the solid support. Preferred reporter groups include those groups recited above.

- The detection reagent is then incubated with the immobilized antibody-polypeptide complex for an amount of time sufficient to detect the bound polypeptide.
- 15 An appropriate amount of time may generally be determined by assaying the level of binding that occurs over a period of time. Unbound detection reagent is then removed and bound detection reagent is detected using the reporter group. The method employed for detecting the reporter group depends upon the nature of the reporter group. For radioactive groups, scintillation counting or autoradiographic methods are
- 20 generally appropriate. Spectroscopic methods may be used to detect dyes, luminescent groups and fluorescent groups. Biotin may be detected using avidin, coupled to a different reporter group (commonly a radioactive or fluorescent group or an enzyme). Enzyme reporter groups may generally be detected by the addition of substrate (generally for a specific period of time), followed by spectroscopic or other analysis of
- 25 the reaction products.

- To determine the presence or absence of a cancer, such as breast cancer, the signal detected from the reporter group that remains bound to the solid support is generally compared to a signal that corresponds to a predetermined cut-off value. In one preferred embodiment, the cut-off value for the detection of a cancer is the average
- 30 mean signal obtained when the immobilized antibody is incubated with samples from patients without the cancer. In general, a sample generating a signal that is three

standard deviations above the predetermined cut-off value is considered positive for the cancer. In an alternate preferred embodiment, the cut-off value is determined using a Receiver Operator Curve, according to the method of Sackett *et al.*, *Clinical Epidemiology: A Basic Science for Clinical Medicine*, Little Brown and Co., 1985, p. 106-7. Briefly, in this embodiment, the cut-off value may be determined from a plot of pairs of true positive rates (*i.e.*, sensitivity) and false positive rates (100%-specificity) that correspond to each possible cut-off value for the diagnostic test result. The cut-off value on the plot that is the closest to the upper left-hand corner (*i.e.*, the value that encloses the largest area) is the most accurate cut-off value, and a sample generating a signal that is higher than the cut-off value determined by this method may be considered positive. Alternatively, the cut-off value may be shifted to the left along the plot, to minimize the false positive rate, or to the right, to minimize the false negative rate. In general, a sample generating a signal that is higher than the cut-off value determined by this method is considered positive for a cancer.

In a related embodiment, the assay is performed in a flow-through or strip test format, wherein the binding agent is immobilized on a membrane, such as nitrocellulose. In the flow-through test, polypeptides within the sample bind to the immobilized binding agent as the sample passes through the membrane. A second, labeled binding agent then binds to the binding agent-polypeptide complex as a solution containing the second binding agent flows through the membrane. The detection of bound second binding agent may then be performed as described above. In the strip test format, one end of the membrane to which binding agent is bound is immersed in a solution containing the sample. The sample migrates along the membrane through a region containing second binding agent and to the area of immobilized binding agent. Concentration of second binding agent at the area of immobilized antibody indicates the presence of a cancer. Typically, the concentration of second binding agent at that site generates a pattern, such as a line, that can be read visually. The absence of such a pattern indicates a negative result. In general, the amount of binding agent immobilized on the membrane is selected to generate a visually discernible pattern when the biological sample contains a level of polypeptide that would be sufficient to generate a positive signal in the two-antibody sandwich assay, in the format discussed above.

Preferred binding agents for use in such assays are antibodies and antigen-binding fragments thereof. Preferably, the amount of antibody immobilized on the membrane ranges from about 25 ng to about 1 μ g, and more preferably from about 50 ng to about 500 ng. Such tests can typically be performed with a very small amount of biological sample.

Of course, numerous other assay protocols exist that are suitable for use with the tumor proteins or binding agents of the present invention. The above descriptions are intended to be exemplary only. For example, it will be apparent to those of ordinary skill in the art that the above protocols may be readily modified to use breast tumor polypeptides to detect antibodies that bind to such polypeptides in a biological sample. The detection of such breast tumor protein specific antibodies may correlate with the presence of a cancer.

A cancer may also, or alternatively, be detected based on the presence of T cells that specifically react with a breast tumor protein in a biological sample. Within certain methods, a biological sample comprising CD4⁺ and/or CD8⁺ T cells isolated from a patient is incubated with a breast tumor polypeptide, a polynucleotide encoding such a polypeptide and/or an APC that expresses at least an immunogenic portion of such a polypeptide, and the presence or absence of specific activation of the T cells is detected. Suitable biological samples include, but are not limited to, isolated T cells. For example, T cells may be isolated from a patient by routine techniques (such as by Ficoll/Hypaque density gradient centrifugation of peripheral blood lymphocytes). T cells may be incubated *in vitro* for 2-9 days (typically 4 days) at 37°C with polypeptide (e.g., 5 - 25 μ g/ml). It may be desirable to incubate another aliquot of a T cell sample in the absence of breast tumor polypeptide to serve as a control. For CD4⁺ T cells, activation is preferably detected by evaluating proliferation of the T cells. For CD8⁺ T cells, activation is preferably detected by evaluating cytolytic activity. A level of proliferation that is at least two fold greater and/or a level of cytolytic activity that is at least 20% greater than in disease-free patients indicates the presence of a cancer in the patient.

As noted above, a cancer may also, or alternatively, be detected based on the level of mRNA encoding a breast tumor protein in a biological sample. For

example, at least two oligonucleotide primers may be employed in a polymerase chain reaction (PCR) based assay to amplify a portion of a breast tumor cDNA derived from a biological sample, wherein at least one of the oligonucleotide primers is specific for (i.e., hybridizes to) a polynucleotide encoding the breast tumor protein. The amplified
5 cDNA is then separated and detected using techniques well known in the art, such as gel electrophoresis. Similarly, oligonucleotide probes that specifically hybridize to a polynucleotide encoding a breast tumor protein may be used in a hybridization assay to detect the presence of polynucleotide encoding the tumor protein in a biological sample.

To permit hybridization under assay conditions, oligonucleotide primers
10 and probes should comprise an oligonucleotide sequence that has at least about 60%, preferably at least about 75% and more preferably at least about 90%, identity to a portion of a polynucleotide encoding a breast tumor protein that is at least 10 nucleotides, and preferably at least 20 nucleotides, in length. Preferably, oligonucleotide primers and/or probes hybridize to a polynucleotide encoding a
15 polypeptide described herein under moderately stringent conditions, as defined above. Oligonucleotide primers and/or probes which may be usefully employed in the diagnostic methods described herein preferably are at least 10-40 nucleotides in length. In a preferred embodiment, the oligonucleotide primers comprise at least 10 contiguous nucleotides, more preferably at least 15 contiguous nucleotides, of a DNA molecule
20 having a sequence recited in SEQ ID NO:1-____. Techniques for both PCR based assays and hybridization assays are well known in the art (*see*, for example, Mullis *et al.*, *Cold Spring Harbor Symp. Quant. Biol.*, 51:263, 1987; Erlich ed., *PCR Technology*, Stockton Press, NY, 1989).

One preferred assay employs RT-PCR, in which PCR is applied in
25 conjunction with reverse transcription. Typically, RNA is extracted from a biological sample, such as biopsy tissue, and is reverse transcribed to produce cDNA molecules. PCR amplification using at least one specific primer generates a cDNA molecule, which may be separated and visualized using, for example, gel electrophoresis. Amplification may be performed on biological samples taken from a test patient and
30 from an individual who is not afflicted with a cancer. The amplification reaction may be performed on several dilutions of cDNA spanning two orders of magnitude. A two-

fold or greater increase in expression in several dilutions of the test patient sample as compared to the same dilutions of the non-cancerous sample is typically considered positive.

In another embodiment, the compositions described herein may be used
5 as markers for the progression of cancer. In this embodiment, assays as described above for the diagnosis of a cancer may be performed over time, and the change in the level of reactive polypeptide(s) or polynucleotide(s) evaluated. For example, the assays may be performed every 24-72 hours for a period of 6 months to 1 year, and thereafter performed as needed. In general, a cancer is progressing in those patients in whom the
10 level of polypeptide or polynucleotide detected increases over time. In contrast, the cancer is not progressing when the level of reactive polypeptide or polynucleotide either remains constant or decreases with time.

Certain *in vivo* diagnostic assays may be performed directly on a tumor. One such assay involves contacting tumor cells with a binding agent. The bound
15 binding agent may then be detected directly or indirectly via a reporter group. Such binding agents may also be used in histological applications. Alternatively, polynucleotide probes may be used within such applications.

As noted above, to improve sensitivity, multiple breast tumor protein markers may be assayed within a given sample. It will be apparent that binding agents
20 specific for different proteins provided herein may be combined within a single assay. Further, multiple primers or probes may be used concurrently. The selection of tumor protein markers may be based on routine experiments to determine combinations that results in optimal sensitivity. In addition, or alternatively, assays for tumor proteins provided herein may be combined with assays for other known tumor antigens.

25 DIAGNOSTIC KITS

The present invention further provides kits for use within any of the above diagnostic methods. Such kits typically comprise two or more components necessary for performing a diagnostic assay. Components may be compounds, reagents, containers and/or equipment. For example, one container within a kit may
30 contain a monoclonal antibody or fragment thereof that specifically binds to a breast

tumor protein. Such antibodies or fragments may be provided attached to a support material, as described above. One or more additional containers may enclose elements, such as reagents or buffers, to be used in the assay. Such kits may also, or alternatively, contain a detection reagent as described above that contains a reporter group suitable
5 for direct or indirect detection of antibody binding.

Alternatively, a kit may be designed to detect the level of mRNA encoding a breast tumor protein in a biological sample. Such kits generally comprise at least one oligonucleotide probe or primer, as described above, that hybridizes to a polynucleotide encoding a breast tumor protein. Such an oligonucleotide may be used,
10 for example, within a PCR or hybridization assay. Additional components that may be present within such kits include a second oligonucleotide and/or a diagnostic reagent or container to facilitate the detection of a polynucleotide encoding a breast tumor protein.

The following Examples are offered by way of illustration and not by way of limitation.

EXAMPLE 1

IDENTIFICATION OF BREAST TUMOR PROTEIN CDNAS

5 This Example illustrates the identification of cDNA molecules encoding breast tumor proteins.

 Potential breast-specific genes present in the GenBank human EST database were identified by electronic subtraction (similar to that described by Vasmatizis et al., *Proc. Natl. Acad. Sci. USA* 95:300-304, 1998). The sequences of EST
10 clones (26,074) derived from various human breast cDNA libraries were obtained from the GenBank public human EST database. Each breast EST sequence was used as a query sequence in a BLASTN (National Center for Biotechnology Information) search against the human EST database. All matches considered identical (BLAST score > 40, length of matching sequence >100 base pairs, density of identical matches over this
15 region > 80%) were grouped (aligned) together in a cluster. Clusters containing more than 200 ESTs were discarded since they probably represented repetitive elements or highly expressed genes such as those for ribosomal proteins. If two or more clusters shared common ESTs, those clusters were grouped together into a "supercluster," resulting in 9,974 breast superclusters.

20 Records for the 479 human cDNA libraries represented in the GenBank release were downloaded to create a database of these cDNA library records. These 479 cDNA libraries were grouped into three groups, Plus (normal breast and breast tumor libraries, and breast cell lines, in which expression was desired), Minus (libraries from other normal adult tissues, in which expression was not desirable), and Other
25 (fetal tissue, infant tissue, ovary, pregnant uterus, male-specific tissues, non-breast tumors and cell lines other than breast cell lines, in which expression was considered to be irrelevant). A summary of these library groups is presented in Table I.

TABLE I**BREAST CDNA LIBRARIES**

Library	# of Libraries
Plus	11
Normal	6
Tumor	4
Cell lines	1
Minus	171
Other	297

Each supercluster was analyzed in terms of the ESTs within the supercluster. The tissue source of each EST clone was noted and used to classify the superclusters into four groups: Type 1 - EST clones derived from the Plus group libraries only; no EST clones derived from Minus or Other group libraries; Type 2 - EST clones derived from the Plus and Other group libraries only; no EST clones derived from the Minus group; Type 3 - EST clones derived from the Plus, Minus and Other group libraries, but the number of EST clones derived from the Plus group is higher than from either the Minus or Other groups; and Type 4 - EST clones derived from Plus, Minus and Other group libraries, but the number of clones derived from the Plus group is higher than the number from the Minus group. For each type, subcategories were generated for clusters containing one or two clones. Types 1a, 2a, 3a and 4a represent Type 1, 2, 3 and 4 clusters where the number of clones in the Plus group is 2. Types 1b, 2b, 3b and 4b represent Type 1, 2, 3 and 4 clusters where the number of clones in the Plus group is 1. This analysis identified 3230 breast clusters. From these clusters, 2501 EST clones were ordered from Research Genetics, Inc. (Huntsville, AL), and were received as frozen glycerol stocks in 96-well plates. A summary of the clusters generated and clones ordered is shown in Table II.

TABLE II**BREAST CLUSTER SUMMARY**

Type	# of Superclusters	# of ESTs Ordered
1	50	49
1a (2/0/0)	130	130
1b (1/0/0)	65	65
2	167	162
2a (2/0/n)	312	321
2a (1/0/n)	1875	1752
3	56	22
3a	32	0
4	333	0
4a	210	0
Total	3230	2501

The EST clone inserts were PCR-amplified for Synteni microarray analysis using amino-linked PCR primers. When more than one PCR product was obtained for a particular clone, that clone was not used for expression analysis. In total, 1896 clones from the electronic subtraction method were analyzed by microarray analysis to identify electronic subtraction breast clones that had high tumor vs. normal tissue mRNA. Such screens were performed using a Synteni (Palo Alto, CA) microarray, according to the manufacturer's instructions (and essentially as described by Schena et al., *Proc. Natl. Acad. Sci. USA* 93:10614-10619, 1996 and Heller et al., *Proc. Natl. Acad. Sci. USA* 94:2150-2155, 1997). Within these analyses, the clones were arrayed on the chip, which was then probed with fluorescent probes generated from normal and tumor breast cDNA, as well as various other normal tissues. The slides were scanned and the fluorescence intensity was measured.

Clones with an expression ratio greater than 2 (*i.e.*, the level in breast tumor cDNA was at least twice the level in normal breast cDNA) were identified as

breast tumor-specific sequences (Table III). The sequences of these clones are provided in SEQ ID NOs:1-124.

TABLE III

5

BREAST-TUMOR SPECIFIC CLONES

SEQ ID NO.	Sequence Designation
1	19702
2	19703
3	B1003C
4	B1002C
5	19708
6	19709
7	19710
8	19711
9	B1006C
10	B1007C
11	19714
12	19715
13	19716
14	19717
15	19718
16	19719
17	19720
18	19721
19	19997
20	19998
21	19999
22	20000
23	20001
24	20002
25	20005
26	20006
27	20007
28	20008
29	20009
30	20010
31	20011
32	20012
33	20013
34	20014

35	20079
36	20080
37	20081
38	20082
39	20083
40	20085
41	20086
42	20087
43	20088
44	20089
45	20090
46	20091
47	20092
48	20093
49	20115
50	20116
51	20118
52	20119
53	20120
54	20121
55	20122
56	20123
57	20124
58	20125
59	20360
60	20361
61	20362
62	22180
63	22181
64	22182
65	22183
66	22185
67	22186
68	22188
69	22189
70	22190
71	22191
72	22192
73	22193
74	22194
75	22196
76	22197
77	22198
78	22199
79	22200
80	22201

81	22202
82	22204
83	22206
84	22207
85	22208
86	22209
87	22210
88	22211
89	22212
90	22213
91	22214
92	22215
93	22216
94	22217
95	22218
96	22219
97	22220
98	22221
99	22222
100	22223
101	22224
102	22225
103	22226
104	22227
105	22228
106	22229
107	22230
108	22231
109	22232
110	22233
111	22234
112	22235
113	22236
114	22237
115	22238
116	22239
117	22240
118	22241
119	22242
120	22243
121	22244
122	22245
123	22334
124	22335

The B1002C sequence (SEQ ID NO:4; 517 bp) was used in a BlastN search of the GenBank Human EST database to identify overlapping sequences that extended further toward the 5' end of the corresponding gene. Two human EST clones were identified in this manner and were purchased from Genome Systems (St. Louis, MO) and sequenced. The resulting sequence information gave rise to a partial predicted open reading frame of 214 amino acids, which is 88% identical over 180 amino acids to the mouse "iroquois homeobox protein 3" (GenBank protein accession #Y15001). The extended B1002C sequence, along with the predicted open reading frame, are provided herein as SEQ ID NOs:125 and 126, respectively. The alignment between B1002C and the mouse iroquois homeobox protein 3 is presented in Figure 1.

EXAMPLE 2

SYNTHESIS OF POLYPEPTIDES

15

Polypeptides may be synthesized on a Perkin Elmer/Applied Biosystems Division 430A peptide synthesizer using Fmoc chemistry with HPTU (O-Benzotriazole-N,N,N',N'-tetramethyluronium hexafluorophosphate) activation. A Gly-Cys-Gly sequence may be attached to the amino terminus of the peptide to provide a method of conjugation, binding to an immobilized surface, or labeling of the peptide. Cleavage of the peptides from the solid support may be carried out using the following cleavage mixture: trifluoroacetic acid:ethanedithiol:thioanisole:water:phenol (40:1:2:2:3). After cleaving for 2 hours, the peptides may be precipitated in cold methyl-t-butyl-ether. The peptide pellets may then be dissolved in water containing 0.1% trifluoroacetic acid (TFA) and lyophilized prior to purification by C18 reverse phase HPLC. A gradient of 0%-60% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) may be used to elute the peptides. Following lyophilization of the pure fractions, the peptides may be characterized using electrospray or other types of mass spectrometry and by amino acid analysis.

30

From the foregoing it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is claimed:

1. An isolated polypeptide, comprising at least an immunogenic portion of a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

(a) sequences recited in SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125;

(b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, under moderately stringent conditions; and

(c) complements of sequences of (a) or (b).

2. An isolated polypeptide according to claim 1, wherein the polypeptide comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, or a complement of any of the foregoing polynucleotide sequences.

3. An isolated polypeptide comprising a sequence recited in SEQ ID NO:126.

4. An isolated polynucleotide encoding at least 15 amino acid residues of a breast tumor protein, or a variant thereof that differs in one or more substitutions, deletions, additions and/or insertions such that the ability of the variant to react with antigen-specific antisera is not substantially diminished, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ

ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, or a complement of any of the foregoing sequences.

5. An isolated polynucleotide encoding a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide comprising a sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, or a complement of any of the foregoing sequences.

6. An isolated polynucleotide, comprising a sequence recited in any one of SEQ ID NOs: 1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125.

7. An isolated polynucleotide, comprising a sequence that hybridizes to a sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, under moderately stringent conditions.

8. An isolated polynucleotide complementary to a polynucleotide according to any one of claims 4-7.

9. An expression vector, comprising a polynucleotide according to any one of claims claim 4-8.

10. A host cell transformed or transfected with an expression vector according to claim 9.

11. An isolated antibody, or antigen-binding fragment thereof, that specifically binds to a breast tumor protein that comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, or a complement of any of the foregoing polynucleotide sequences.

12. A fusion protein, comprising at least one polypeptide according to claim 1.

13. A fusion protein according to claim 12, wherein the fusion protein comprises an expression enhancer that increases expression of the fusion protein in a host cell transfected with a polynucleotide encoding the fusion protein.

14. A fusion protein according to claim 12, wherein the fusion protein comprises a T helper epitope that is not present within the polypeptide of claim 1.

15. A fusion protein according to claim 12, wherein the fusion protein comprises an affinity tag.

16. An isolated polynucleotide encoding a fusion protein according to claim 12.

17. A pharmaceutical composition, comprising a physiologically acceptable carrier and at least one component selected from the group consisting of:

- (a) a polypeptide according to claim 1;
- (b) a polynucleotide according to claim 4;
- (c) an antibody according to claim 11;
- (d) a fusion protein according to claim 12; and
- (e) a polynucleotide according to claim 16.

18. A vaccine comprising an immunostimulant and at least one component selected from the group consisting of:

- (a) a polypeptide according to claim 1;
- (b) a polynucleotide according to claim 4;
- (c) an antibody according to claim 11;
- (d) a fusion protein according to claim 12; and
- (e) a polynucleotide according to claim 16.

19. A vaccine according to claim 18, wherein the immunostimulant is an adjuvant.

20. A vaccine according to any claim 18, wherein the immunostimulant induces a predominantly Type I response.

21. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a pharmaceutical composition according to claim 17.

22. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a vaccine according to claim 18.

23. A pharmaceutical composition comprising an antigen-presenting cell that expresses a polypeptide according to claim 1, in combination with a pharmaceutically acceptable carrier or excipient.

24. A pharmaceutical composition according to claim 23, wherein the antigen presenting cell is a dendritic cell or a macrophage.

25. A vaccine comprising an antigen-presenting cell that expresses a polypeptide comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (a) sequences recited in SEQ ID NOs:1-125;
- (b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-125 under moderately stringent conditions; and
- (c) complements of sequences of (i) or (ii);
in combination with an immunostimulant.

26. A vaccine according to claim 25, wherein the immunostimulant is an adjuvant.

27. A vaccine according to claim 25, wherein the immunostimulant induces a predominantly Type I response.

28. A vaccine according to claim 25, wherein the antigen-presenting cell is a dendritic cell.

29. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of an antigen-presenting cell that expresses a polypeptide comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (a) sequences recited in SEQ ID NOs:1-125;
- (b) sequences that hybridize to a sequence recited in any one of SEQ ID NOs:1-125 under moderately stringent conditions; and
- (c) complements of sequences of (i) or (ii) encoded by a polynucleotide recited in any one of SEQ ID NOs:1-125;

and thereby inhibiting the development of a cancer in the patient.

30. A method according to claim 29, wherein the antigen-presenting cell is a dendritic cell.

31. A method according to any one of claims 21, 22 and 29, wherein the cancer is breast cancer.

32. A method for removing tumor cells from a biological sample, comprising contacting a biological sample with T cells that specifically react with a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (i) polynucleotides recited in any one of SEQ ID NOs:1-125; and
- (ii) complements of the foregoing polynucleotides;

wherein the step of contacting is performed under conditions and for a time sufficient to permit the removal of cells expressing the antigen from the sample.

33. A method according to claim 32, wherein the biological sample is blood or a fraction thereof.

34. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient a biological sample treated according to the method of claim 32.

35. A method for stimulating and/or expanding T cells specific for a breast tumor protein, comprising contacting T cells with at least one component selected from the group consisting of:

(a) polypeptides comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (i) sequences recited in SEQ ID NOs:1-125;
- (ii) sequences that hybridize to a sequence recited in any one of SEQ

ID NOs:1-125 under moderately stringent conditions; and

- (iii) complements of sequences of (i) or (ii);

(b) polynucleotides encoding a polypeptide of (a); and

(c) antigen presenting cells that express a polypeptide of (a);

under conditions and for a time sufficient to permit the stimulation and/or expansion of T cells.

36. An isolated T cell population, comprising T cells prepared according to the method of claim 35.

37. A method for inhibiting the development of a cancer in a patient, comprising administering to a patient an effective amount of a T cell population according to claim 36.

38. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) polypeptides comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (1) sequences recited in SEQ ID NOs:1-125;
- (2) sequences that hybridize to a sequence recited in any one of

SEQ ID NOs:1-125 under moderately stringent conditions; and

- (3) complements of sequences of (1) or (2);
- (ii) polynucleotides encoding a polypeptide of (i); and
- (iii) antigen presenting cells that expresses a polypeptide of (i);

such that T cells proliferate; and

(b) administering to the patient an effective amount of the proliferated T cells, and thereby inhibiting the development of a cancer in the patient.

39. A method for inhibiting the development of a cancer in a patient, comprising the steps of:

(a) incubating CD4⁺ and/or CD8⁺ T cells isolated from a patient with at least one component selected from the group consisting of:

(i) polypeptides comprising at least an immunogenic portion of a breast tumor protein, or a variant thereof, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence selected from the group consisting of:

- (1) sequences recited in SEQ ID NOs:1-125;
- (2) sequences that hybridize to a sequence recited in any one of

SEQ ID NOs:1-125 under moderately stringent conditions; and

- (3) complements of sequences of (1) or (2);
- (ii) polynucleotides encoding a polypeptide of (i); and
- (iii) antigen presenting cells that express a polypeptide of (i);

such that T cells proliferate;

(b) cloning at least one proliferated cell to provide cloned T cells; and

(c) administering to the patient an effective amount of the cloned T cells, and thereby inhibiting the development of a cancer in the patient.

40. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with a binding agent that binds to a breast tumor protein, wherein the tumor protein comprises an amino acid

sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-125 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of polypeptide that binds to the binding agent; and

(c) comparing the amount of polypeptide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

41. A method according to claim 40, wherein the binding agent is an antibody.

42. A method according to claim 43, wherein the antibody is a monoclonal antibody.

43. A method according to claim 40, wherein the cancer is breast cancer.

44. A method for monitoring the progression of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient at a first point in time with a binding agent that binds to a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1-125 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of polypeptide that binds to the binding agent;

(c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and

(d) comparing the amount of polypeptide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

45. A method according to claim 44, wherein the binding agent is an antibody.

46. A method according to claim 45, wherein the antibody is a monoclonal antibody.

47. A method according to claim 44, wherein the cancer is a breast cancer.

48. A method for determining the presence or absence of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NO:1-125 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide; and

(c) comparing the amount of polynucleotide that hybridizes to the oligonucleotide to a predetermined cut-off value, and therefrom determining the presence or absence of a cancer in the patient.

49. A method according to claim 48, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

50. A method according to claim 48, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.

51. A method for monitoring the progression of a cancer in a patient, comprising the steps of:

(a) contacting a biological sample obtained from a patient with an oligonucleotide that hybridizes to a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide

sequence recited in any one of SEQ ID NO:1-125 or a complement of any of the foregoing polynucleotide sequences;

(b) detecting in the sample an amount of a polynucleotide that hybridizes to the oligonucleotide;

(c) repeating steps (a) and (b) using a biological sample obtained from the patient at a subsequent point in time; and

(d) comparing the amount of polynucleotide detected in step (c) to the amount detected in step (b) and therefrom monitoring the progression of the cancer in the patient.

52. A method according to claim 51, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a polymerase chain reaction.

53. A method according to claim 51, wherein the amount of polynucleotide that hybridizes to the oligonucleotide is determined using a hybridization assay.

54. A diagnostic kit, comprising:

(a) one or more antibodies according to claim 11; and

(b) a detection reagent comprising a reporter group.

55. A kit according to claim 54, wherein the antibodies are immobilized on a solid support.

56. A kit according to claim 54, wherein the detection reagent comprises an anti-immunoglobulin, protein G, protein A or lectin.

57. A kit according to claim 54, wherein the reporter group is selected from the group consisting of radioisotopes, fluorescent groups, luminescent groups, enzymes, biotin and dye particles.

58. An oligonucleotide comprising 10 to 40 contiguous nucleotides that hybridize under moderately stringent conditions to a polynucleotide that encodes a breast tumor protein, wherein the tumor protein comprises an amino acid sequence that is encoded by a polynucleotide sequence recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125, or a complement of any of the foregoing polynucleotides.

59. A oligonucleotide according to claim 58, wherein the oligonucleotide comprises 10-40 contiguous nucleotides recited in any one of SEQ ID NOs:1, 3, 4, 8, 9, 11, 12, 14, 15, 17, 26, 30, 31, 35, 36, 47, 52, 55, 60, 62, 63, 66, 69, 71, 72, 74-78, 81, 83-87, 92, 94, 97, 99-104, 107, 108, 110, 112, 113, 117, 118, 120-122 or 125.

60. A diagnostic kit, comprising:

(a) an oligonucleotide according to claim 59; and

(b) a diagnostic reagent for use in a polymerase chain reaction or hybridization assay.

SEQUENCE LISTING

<110> Corixa Corporation
 Mitcham, Jennifer L.
 Jiang, Yuqiu

<120> COMPOSITIONS AND METHODS FOR THE THERAPY
 AND DIAGNOSIS OF BREAST CANCER

<130> 210121.482PC

<140> PCT

<141> 2000-06-23

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<212> DNA

<213> Homo sapien

<400> 16

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ggggaggagg acctgtgtgt caggctctgc catgggaacg agtgtaaacc gtggctgtct      420
cctgcagtga gccaccgcgg caggcacgtt gactgtttta ctgacatcac tcaaaagcta      480
aagcaataac attctcttgc gttgctgagt cagctgttca tttgtccgcc agctcctgga      540
ctggatgtgt gaaaggcatc acatttccat tttcctccgt gtaaatgttt tatgtgttcg      600
cctactgatc ccattcgttg cttctattgt aaatatttgt catttgtatt tattatctct      660
gtgttttccc cctaaggcat aaaatggttt actgtgttca tttgaaccca aaaaaaaaaa      720
aaaaaaaaaa aa                                                              732

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<210> 18

<211> 103

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(103)

<223> n = A,T,C or G

<400> 18

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cnagggggccc ggggcggggt ccgccggccc tgcggggccgc cggtgaaata ccactactct      60
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<210> 19

<211> 586

<212> DNA

<213> Homo sapien

<400> 19

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acgcctgcag gtaccggtcc ggaattcccg ggtcgactac tactactaaa ttcgcggccg      60
cgtcgactcc cagcccctat cgagaaaacc atctccaaag ccaaaggcca gccccgagaa      120
ccacagggtg acaccctgcc cccatcccgg gaggagatga ccaagaacca ggtcagcctg      180
acctgcctgg tcaaaggctt ctatcccagc gacatcgccg tggagtggga gagcaatggg      240
cagccggaga acaactacaa gaccacgcct cccgtgctgg actccgacgg ctcttcttc      300
ctctatagca agctcaccgt ggacaagagc aggtggcagc aggggaacgt cttctcatgc      360
tccgtgatgc atgaggctct gcacaaccac tacacgcaga agagcctctc cctgtccccg      420
ggtaaatgag tgcgacggcc ggcaagcccc cgtcccccg gctctcgagg tcgcacgagg      480
atgcttggca cgtaccccgt ctacatactt cccaggcacc cagcatggaa ataaagcacc      540
caccactgcc ctggaaaaaa gtcgacgcgg ccgcgaattt agtagt                                                              586

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<210> 20

<211> 646

<212> DNA

<213> Homo sapien

<400> 20

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ccgggtcgac tactactact aaattcgcgg ccgcgtcgac caacagggga gagtgttaga      60
gggagaagtg cccctgttg aagctctttg tgatgggcga gctcaggccc tgatgggtga      120
cttcgcgggc gttagacttg tgtttctcgt agtctgcttt gctcagcgtc aggggtgctgc      180
tgaggctgta ggtgctgtcc ttgctgtcct gctctgtgac actctcctgg gatttaccgc      240

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attggaggggc	gttatccacc	ttccactgta	ctttggcctc	tctgggatag	aagttattca	300
gcagggcacac	aacagaggca	gttccagatt	tcaactgctc	atcagatggc	gggaagatga	360
agacagatgg	tgcagccaca	gttcgtttga	tctccagctt	ggccccctgg	ccaaaagtgt	420
acggaggtga	gctgtcgacg	cggccgcgaa	tttagtagta	gtaggcggcc	gctctagagg	480
atccaagctt	acgtacgcgt	gcatgcgacg	tcatagctct	tctatagtgt	cacctaaatt	540
caattcactg	gccgtcgttt	tacaacgtcg	tgactgggaa	aaccctggcg	ttacccaact	600
taatcgctt	gcagcacatc	cccccttcgc	cagctggcgt	aatagc		646

<210> 21

<211> 625

<212> DNA

<213> Homo sapien

<400> 21

tttttttttt	tttaccaaaa	ttgaccttta	tttgttgtac	taaagcctgt	ttaacttttg	60
atacaaagta	acatttttagt	acagaaaatc	ccagtctgtc	agctcagtag	ctgtctgtgc	120
acactgtacc	atctcagtc	cactctgcct	gtaacttaaa	aaacagcccc	tacccccaga	180
gggtctgcga	gttaatacct	tgagaatagt	ctacagtttt	tcatagttag	tctgagctag	240
aaaacttgta	cctgtaaaac	aaaggacagc	attgaggact	gaaacttgct	tytttttttg	300
aacaactgtg	caagaaaata	tatccctttt	taaaaaacat	cagttatggc	taaactacaa	360
tctagtgtct	agaattacaa	agaataaaat	gaaatcaaag	atttctcgct	agtaaaatga	420
aatgttagga	acagtattaa	aatataggtc	ctaccccaac	gacacttaca	cagagcccag	480
tacagtacct	attattaaca	ggcgcgatag	cttaaggagg	aaccacatca	aatcttcagc	540
cagacatatc	tagcctcaga	agtgcacaaa	aaaaaaaaag	cccccaacg	aagacacccc	600
actgagtagg	gtgcatgccg	tgagt				625

<210> 22

<211> 576

<212> DNA

<213> Homo sapien

<400> 22

ctctcagttc	ctgcaagcag	ctgagcgcta	tggcattaac	accactgaca	tcttccaaac	60
tgtggacctc	tgggaaggaa	agaatatggc	ctgtgtgcag	cggacgctga	tgaatctggg	120
tgggctggca	gtagcccgag	atgatgggct	cttctctggg	gatcccaact	ggttccctaa	180
gaaatccaag	gagaatcctc	ggaacttctc	ggataaccag	ctgcaagagg	gcaagaacgt	240
gatcgggtta	cagatgggca	ccaaccgcgg	ggcgtctcag	gcaggcatga	ctggctacgg	300
gatgccacgc	cagatcctct	gatccacccc	caggccttgc	ccctgccctc	ccacgaatgg	360
ttaatatata	tgtagatata	tatttttagca	gtgacattcc	cagagagccc	cagagctctc	420
aagctccttt	ctgtcagggg	gggggggttca	gcctgtcctg	tcacctctga	ggtgcctgct	480
ggcatcctct	cccccatgct	tactaataca	ttcccttccc	catagccatc	aaaactggac	540
caactggcct	cttccttttc	cctgggacca	aaattt			576

<210> 23

<211> 416

<212> DNA

<213> Homo sapien

<400> 23

ggcacgaggg	cggccccgcg	ggtctgtctc	ttgcttcaac	agtgtttgga	cggaaacagat	60
ccggggactc	tcttcagacc	tccgaccgcc	ctccgatttc	ctctccgctt	gcaacctccg	120
ggaccatctt	ctcggccatc	tccgtctctc	gggacctgcc	agcaccgttt	ttgtggtag	180
ctccttcttg	ccaaccaacc	atgagctccc	agattcgtca	gaattattcc	accgacgtgg	240
aggcagccgt	caacagcctg	gtcaatttgt	acctgcagge	ctctacacc	tacctctctc	300
tgggcttcta	tttcggaccg	cgatgatgtg	gctctggaag	gcgtgagcca	cttcttccgc	360

gaactggccg aggagaagcg cgagggctac gagegtctcc tgaagatgca aaacca 416

<210> 24

<211> 1037

<212> DNA

<213> Homo sapien

<400> 24

gctttaccta	tggtggttgt	tatggcaaca	agaacaactt	tgaggaagag	cwttagtgcc	60
tcgagtcctt	tcgcggcatc	tccattaagg	atgtgtttgg	cctgaggcgg	gaaatcccca	120
ttcccagcac	aggctctgtg	gagatggctg	tcgcagtgtt	cctggtcac	tgcatgtgg	180
tggtggtagc	catcttgggt	tactgcttct	tcaagaacca	gagaaaggac	ttccacggac	240
accaccacca	cccaccacc	acccttgcca	gtccactgt	ctccactacc	gaggacacgg	300
agcacctggt	ctataaccac	accaccggc	ccctctgagc	ctgggtctca	ccggctctca	360
cctggccctg	cttctctgct	gccaaggcag	aggcctgggc	tgggaaaaac	tttggaaacca	420
gactcttgcc	tgtttccag	gcccactgtg	cctcagagac	cagggtccca	gcccccttg	480
gagaagtctc	agcctaagctc	acgtcctgag	aaagctcaaa	ggtttggaag	gagcagaaaa	540
cccttggggc	agaagtacca	gactagatgg	acctgcctgc	ataggagttt	ggagggaagt	600
ggagttttgt	ttctctgtt	caaagctgcc	tgtccctacc	ccatggtgct	aggaagagga	660
gtggggtggt	gtcagaccct	ggaggcccca	accctgtcct	cccagactcc	tyttccatgc	720
tgtagcgcca	gggctgggag	gaaggacttc	cctgtgtagt	ttgtgctgta	aagagtgtgt	780
ttttgtttat	ttaatgctgt	ggcatgggtg	aagaggagg	gaagaggcct	gtttggccty	840
tytgtcctyt	ytctctcttc	cccccaagatt	gagctytytg	cccttgatca	gccccmccct	900
ggcctagacc	agcagacaga	gccaggagag	gstcagctgc	attccgcagc	ccccaccccc	960
aagggttttc	aacatcacag	cccagccac	ccactgggta	ataaaagtgg	tttgggaaaa	1020
aaaaaaaaaa	aaaaaaaa					1037

<210> 25

<211> 1144

<212> DNA

<213> Homo sapien

<400> 25

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gaccgtcagg	gagaaaaatg	ccakatgatg	agctgcacat	gtcttgggaa	cggaaaaagga	120
gaattcaagt	gtgaccctca	tgaggcaacg	tgttacgatg	atgggaagac	ataccacgta	180
ggagaacagt	ggcagaagga	atatctcgg	gccatttgct	cctgcacatg	ctttggaggc	240
cagcggggct	ggcgtgtga	caactgccgc	agacctgggg	gtgaaccacg	tcccgaaaggc	300
actactggcc	agtcctacaa	ccagtattct	cagagatacc	atcagagaa	aaacactaat	360
gttaattgcc	caattgagt	cttcatgcct	ttagatgtac	aggctgacag	agaagattcc	420
cgagagtaaa	tcattcttcc	aatccagagg	aacaagcatg	tctctctgcc	aagatccatc	480
taaactggag	tgatgttagc	agaccagct	tagagtctct	ctttctttct	taagcccttt	540
gctctggagg	aagttctcca	gcttcagctc	aactcacagc	ttctccaagc	atcaccttgg	600
gagtttctct	agggttttct	cataaatgag	ggctgcacat	tgctgttct	gcttcgaagt	660
attcaatacc	gtcagttatt	ttaaatgaag	tgattctaa	atttggtttg	ggatcaatag	720
gaaagcatat	gcagccaacc	aagatgcaaa	tgttttgaaa	tgatatgacc	aaaatttttaa	780
gtaggaaagt	cacccaaaca	cttctgcttt	cacttaagt	tytgcccgc	aatactgtag	840
gaacaagcat	gatcttggtt	ctgtgatatt	ttaaatatcc	mcagtactca	ctttttccaa	900
atgatcctag	taattgccta	gaaatatctt	tctcttacct	gttattttatc	aatttttccc	960
agtattttta	tacggaaaaa	attgtattga	aaacmcttag	tatgcagttg	ataagaggaa	1020
tttggtataa	ttatggtggg	tgaaaatttt	ttatactgta	tgtgccaaag	ctttaaaact	1080
gtggaaagac	aactgtttta	ataaaagatt	tacattcccc	aacttgaaaa	aaaaaaaaaa	1140
aaaa						1144

<210> 26

<211> 488

<212> DNA

<213> Homo sapien

<400> 26

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gtactcttg	gagtttccat	cttitytggtc	tctgcccaga	atccgacaac	agctgctcca	120
gctgacacgt	atccagctac	tggtcctgct	gatgatgaag	ccctgatgc	tgaaaccact	180
gctgctgcaa	ccactgcgac	cactgctgct	cctaccactg	caaccaccgc	tgcttctacc	240
actgctcgta	aagacattcc	agttttacc	aaatgggttg	gggatctccc	gaatggtaga	300
gtgtgtccct	gagatggaat	cagcttgagt	cttctgcaat	tggtcacaac	tattcatgct	360
tctgtgatt	tcattcaact	acttaccttg	cctacgatat	cccccttatt	tctaatacagt	420
ttatttttyt	tcaaataaaa	aataactatg	agcaacataa	aaaaaaaaaa	aaaaaaaaaa	480
aaaaaaaa						488

<210> 27

<211> 764

<212> DNA

<213> Homo sapien

<400> 27

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attcagggca	tgtctctccc	ccaggcccca	tcttcataca	cttccgctaa	gatgcccaca	120
attcatcccc	cttcatgtgc	agcttgaaaa	accctgacat	tggtgctccc	tgccgggcat	180
acctgccatt	gccactgccc	atctctgtga	tgagggaattc	aacaccagat	ctctggcagc	240
tcttgaggga	ttggccagtg	gccattctca	ggggtgctgt	ctagtcttcc	cagagccccg	300
ccaaggtcct	ggccaccgtg	gcagagcggg	gaggttcccc	cactctaaag	cctactcctt	360
ctctctctag	cctcttcagg	agtggtgtgt	agcagcagca	atttccacat	caatgtagaa	420
gagtcagtg	atggacaggt	ggtttcttcc	cacaagagag	aaatytaagt	gtctattgca	480
ggagaaacgt	cccttgccac	tccccactct	catcaggcca	agtggaggac	tggccagagg	540
gcctgcacat	gcaaaactca	gtccctgcct	tcagagagct	gaaaagggtc	cctcggtctt	600
ttatttcagg	gctttgcatg	cgtctctattc	ccccctctgc	tctccccmcc	ttytttgagg	660
caaggagatg	cagctgtatt	gtgtaacaag	ctcatttgta	cagtgtctgt	tcattgtaata	720
aagaattact	tttctttttg	caaaaaaaaa	aaaaaaaaaa	aaaa		764

<210> 28

<211> 802

<212> DNA

<213> Homo sapien

<400> 28

accatcactt	gccgggcaag	tcagagtatt	aacaactatt	taaattggta	tctgcagaaa	60
ccagggaag	cccctaacct	cctgatctat	gctgcatcca	ctttgcaaag	tggggtccca	120
tcaagggtca	stggcagtga	atctgggaca	gatttcactc	tcaccatcag	cagtctgcaa	180
cctgaagatt	ttgcaactta	ctactgycaa	cagagttaca	ctacccatt	cactttcrgm	240
cctgggacca	aagtggatgt	caaacgaact	gtggctgcac	catctgtctt	catcttcccg	300
ccatctgatg	agcagwtgaa	atctggaact	gcctctgttg	tgtgcctgct	gaataacttc	360
tatcccagag	aggccaaagt	acagtggaag	gtggataacg	ccctccaatc	gggtaactcc	420
caggagagtg	tcacagagca	ggacagcaag	gacagcacct	acagcctcag	cagcaccctg	480
acgtctgagca	aagcagacta	cgagaaacac	aaagtctacg	cctgsgaagt	cacccatcag	540
ggctgagct	cgcccgctac	aaagagcttc	aacaggggag	agtgttagag	ggagaagtgc	600
ccccacctgc	tcctcagttc	cagcctgacc	ccctcccatc	ctttggccty	tgaccctttt	660
tccacagggg	acctaccctt	attgcggtcc	tccagctcat	ctttcacctc	acccccctcc	720
tcctccttgg	ctttaattat	gctaagtgtg	gaggagaatg	aataaataaa	gtgaatcttt	780
gcaaaaaaaaa	aaaaaaaaaa	aa				802

<210> 29
 <211> 620
 <212> DNA
 <213> Homo sapien

<400> 29
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 ctggctagga gaaactatca atgctggcag ccagtttgaa tataatgtag aaggagtgc 120
 ttccaaggaa atggctaccc aacttgctt catgcgcctg ctggccaact atgcctctca 180
 gaacatcacc taccactgca agaacagcat tgcatacatg gatgaggaga ctggcaacct 240
 gaaaaaggct gtcattctac agggctctaa tgatgttgaa cttgttgctg agggcaacag 300
 caggttcact tacactgttc ttgtagatgg ctgtctctaaa aagacaaatg aatggggaaa 360
 gacaatcatt gaatacaaaa caaataagcc atcacgcctg cccttccttg atattgcacc 420
 tttggacatc ggtgggtgctg accaggaatt ctttgtggac attggcccag tctgtttcaa 480
 ataaatgaac tcaatctaaa ttaaaaaaga aagaatttg aaaaaacttt ctctttgcca 540
 tttcttcttc ttctttttta actgaaagct gaatccttcc atttcttctg cacatctact 600
 tgcttaaat gtgggcaaaa 620

<210> 30
 <211> 644
 <212> DNA
 <213> Homo sapien

<400> 30
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 aaccaccmgc cmatttaaca taaaacactt gkcaagctga gtagactgkt ttcttatgkg 120
 aaccacaaaa tattttctct gaaatctaca cttagtttaa aaacagagat gggattttgc 180
 atattagctt gaaaataagt atatgatgat gatattaggk gcccactagc acctagtttt 240
 tacagctttg cattgkcacc ccactactgc cagggaccca gcccaggca tacacagatg 300
 aaaggacagt ttacacttct tggcaaaaac cttcagaaca attgtcaaca tactctcaaa 360
 tgtctttccc actcagaaat gaggagcaag gtgtatgact ttagattcaa gaagtatatg 420
 gggctaaata tctttaaaag tataactctg gacaatgtac ttagggacct actacttact 480
 caaaataggg tagtagcatt aataactaaa tagggcgtga gtcattgagaa agattccctg 540
 catctcttgc caagtattaa cactagtttt actaataagc aacggggtag ataactttga 600
 agattgcac cacttcaaca caaaaaaatg tgaatatccc tcgt 644

<210> 31
 <211> 674
 <212> DNA
 <213> Homo sapien

<400> 31
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 tcaagagtaa tccacacgtg agaaaactaga caatgtacat tcatgtgttc tcttgaaagg 120
 aaaggagag ctgtaagctt cactctgtcc tacaccggag aaaagcagga ataactttac 180
 cgtggaaata atgttttagct tttatcagag aaaattgtcc ttctagagca tagagtccca 240
 aaactcaatt ctgggtttcc cctgtttttt tttttttttt ttttcccaac atatgaactg 300
 cagcatatca ctttttcttt ttgtgcctca ggttcctcag ctgtaaaatt gaaaaatata 360
 tgtattaata atattattaa taataataat ggtaatgtag tacttgtttg taaagcactt 420
 tgagatcctt ggttgaaaag caccatagga gtgccaagta ttattatgtg gccaaagggg 480
 ttatttaaac tgcagttcc caaaggccag gaaagggttg ggtcattttt cttaaagacg 540
 agctgtaaat atcaactagg cagccaatag tgttgactat gaagatgcaa aactattact 600
 aggmtgataa aatcatagtt tcttaatggc taccaataag gcaaatatca caataataaa 660
 caccaaattc cgta 674

<210> 32
 <211> 713
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(713)
 <223> n = A,T,C or G

<400> 32
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 ccttgaaaaa aaactattaa aaaggataga cattttctgat gtaagtaaaa cccccaagca 180
 acttaatatc catctgtcag tcatcaactt tccccctaga tttttttttt aactagttct 240
 gaaagtgtca agaatagctt cctttttcac cctacctagg ttccctcctt gctggcataa 300
 aggccaaagca gtgaaatgaa ccttgggtta gagttatggt aggcaaaaag aaaaggagaa 360
 tttaggaat aaatatgcta taaacaaggt acagtacagt gagagttaac aatcctagga 420
 atcctgcctg tcacaagctc ccagttccct gtcattttat ctcatgatt agaactgac 480
 ttccatttaa ctattacaga aagcagtaac tattgcaact atctaatagt tcacacaaaa 540
 gaattaaaag cttaaaaata attttttagga aacacaatat tcaaaatcta aacacactga 600
 taaattatta agattaagat tatwtatgtg ataaatgaaa tctcctacca atccatccag 660
 cctttaccag ggaagaaaag caattatttc atttcagata gaaatacaaa aaa 713

<210> 33
 <211> 698
 <212> DNA
 <213> Homo sapien

<400> 33
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 tgccagcccc acacagctac agctttcttg ctcccttcag cccccagccc ctccccctc 180
 tcccacctg tacctcatcc catgagaccc tgggtgectg ctctttctgc acccttggac 240
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 atctgtcaac aggggctga ggtcccagga agtggccaaa agctagacag atccccgttc 360
 ctgacatcac agcagcctcc aacacaaggc tccaagacct aggttcctgg acgagatggg 420
 aaggcacagg gagaagggat aacctacac ccagacccca ggctggacat gctgactgtc 480
 ctctccccctc cagccttttg ccttggcttt tctagcctat ttacctgcag gctgagccac 540
 tctcttccct ttccccagca tcaactccca aggaagagcc aatgttttcc acccataatc 600
 ctttctgccc acccctagtt cctctgctc agccaagcta gwtatcagct ttcagggcca 660
 tggttcacat tagaataaaa ggtagtaatt agaaaaaa 698

<210> 34
 <211> 605
 <212> DNA
 <213> Homo sapien

<400> 34
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 cgtttgtgca gccaaacctc cttcacaata gccacgtcgc tcatggtgcc cgaggctccc 120
 gcgacgtca cgcgtctctc tcaggctggc gctccccag cccagctggc ctggccacag 180
 cctctgatgc accagctgac aggetgcctc ctccaggcag cccctttgac ttctttgacc 240
 caggctggct cggccttccc taagcccctg gtgacagatg gccccgtttg ctctccctgt 300

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ccatggtggt cctacccatg gatcctggga cagggcacag ggctcctccc tgctccccag 360
actagggaaag caaagaaatt caaacatgag gaagacagga ccaggatgca ggccactggc 420
gcaaacggga gtccagagcc ctccagcgca agcccaaaaa cctcctggga gaaaccccag 480
gcccctccta aaccacagcg cccctgccgg tctgaatctg gttcattcat ttggccaaca 540
tgtaccgggc gtctcaggtt ttgccaggcc cagtgtctggg cgctggagac aaagagcctc 600
gtrcc 605

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<210> 35
<211> 753
<212> DNA
<213> Homo sapien

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<220>
<221> misc_feature
<222> (1)...(753)
<223> n = A,T,C or G

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<400> 35
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scagtcaaac agcctgagaa agtggcagct accaggcagg agatcttcca ggagcagttg 180
gcagcagtg cagagttccg cggctcttggg cccctcttca agtcctcgcc tgagcccgtg 240
gccctcaccg agtcagagac ggagtatgtc atccgctgca ccaaacacac cttcaccaac 300
cacatgggtt ttcatgttga ctgcacaaac aactcaatg accagacctt ggagaatgtc 360
acagtgcaga tggagcccac tgaggcctat gaggtgtct gttacgtgcc tgcccggagc 420
ctgccctaca accagcccgg gacctgtct acactggtgg cactgcccac agaagacccc 480
acagctgtgg cctgcacatt cagctgcatg atgaagttca ctgtcaagga ctgtgatccc 540
accactgggg agactgatga cgaaggctat gaggatgagt atgtgctgga agatctggaa 600
gttactgtag ctgatcacat tcaaaaggtc atgaaactga acttcgaagc agcctgggat 660
gangtagggg atgaatttga gaaggaggaa acgttcacct tgtctaccat caagacactt 720
gaagaggctg ggggnaatat tgggaagtct tgg 753

```

```

<210> 36
<211> 433
<212> DNA
<213> Homo sapien

```

```

<400> 36
ggggcttgca atcatttgaa ttgttctggt tcacaataaa ggagattcac tgggttctgc 60
attttcagga ttcaatagaa ctgcttcatt aaaaaataa tccttagcaa gcattcgaat 120
cctaactgct ttgatgcact tgccctcggg cactgtcat ttccaatat gtaggtgtca 180
aagtcaaaaag tatttactgg gagaaaaaag agaggagtgg ttgtagaagt ctccctaagt 240
cagacatgtc aagcaatcag ccaacgtggt gtattttctc ttcaatattt tagtgtgaat 300
tgagacactg agataaagac atcgtgcaga gataaatggg gatacagtta aatgtagcaa 360
ctcttgagtt cattttttcc cactgtagca aaattaatgc tttctcttta ttgaaataaa 420
ttgtcattc etc 433

```

```

<210> 37
<211> 601
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(601)

```

<223> n = A,T,C or G

<400> 37

```
aggagtgtccc cggtgtcccc tcacctgtgt gcaagtacat ctctgtcgcc gagtgcctga      60
agttcgaaaa gggccctttt gggaagaact gcagcgcgcc gtgtccgggc ctgcagctgt      120
cgaacaaccc cgtgaagggc aggacctgca aggagagggg ctgagagggc tgctgggtgg      180
cctacacgct ggagcagcag gacgggatgg accgctacct catctatgtg gatgagagcc      240
gagagtgtgt ggagggcccc aacatcgccg ccatcgctcg gggcaccgtg gcaggcatcg      300
tgctgatcgg cattctctgt ctggctcatct ggaaggtctt gatccacctg agcgacctcc      360
gggagtacag gcgcttttag aaggagaagc tcaagtccca gtggaacaat gataatcccc      420
ttttcaagag cgccaccacg acgggtcatga accccaagtt tgctgagagt taggagcact      480
tggtgaaaac aangggccgtc aggacccacc atgtctgtccc atacgcgggc ganacatggc      540
ttggcacact nttgangatg taccatttac caaaatcnan tttttccctt tnaatnanct      600
g                                                                                   601
```

<210> 38

<211> 713

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1) ... (713)

<223> n = A,T,C or G

<400> 38

```
gcttccgcct attgattctg ctcatattata caataatgag gagcagggtg gactggccat      60
ccgaagcaag attgcagatg gcaktgtgaa gagagaagac atattctaca cttcaaaagt      120
ttggtgcaat tcccacgac cagagttggt ccgaccagcc ttggaaaggc cactgaaaaa      180
tcttcaattg gattatgttg acctctacct tattcatttt ccagtgtctg taaagccagg      240
tgaggaaagt atcccaaaag atgaaaatgg aaaaatacta ttgacacag tggatctctg      300
tgccacgttg gaggccgttg agaagtgtaa agatgcagga ttggccaagt ccatcggggt      360
gtccaaacttc aaccgcaggc agctggagat gatcctcaac aagccagggc tcaagtacaa      420
gcctgtctgc aaccaggttg aatgtcatcc ttacttcaac cagagaaaac tgctggattt      480
ctgcaagtca aaagacattg ttctggttgc ctatagtgtc ctgggatccc accgagaaga      540
accatgggtg gaccggaact ccccggtgct cttggaggac ccagtccttt gtgccttggc      600
aaaaaagcac aagcgaaccc cagccctgat tgccctgcgc taccactaca gcgtgggggt      660
gtggtccttg ccaagagcta caatgagcag cgcacacnac agaacgtgca ggg                    713
```

<210> 39

<211> 451

<212> DNA

<213> Homo sapien

<400> 39

```
gaaagaactg actgaaacgt ttgagatgaa gaaagttctc ctctgatca cagccatctt      60
ggcagtggtc gttggtttcc cagtctctca agaccaggaa cgagaaaaaa gaagtatcag      120
tcgacagcga tgaattagct tcagggtttt ttgtgttccc ttaccataat ccatttcgcc      180
cacttccacc aattccattt ccaagatttc catggtttag acgtaatttt cctattccaa      240
tacctgaate tgcccctaca actccccttc ctagcgaaaa gtaaacaaaga aggaaaagtc      300
acgataaacc tggtcacctg aaattgaaat tgagccactt ccttgaagaa tcaaaattcc      360
tgttaataaa agaaaaacaa atgtaattga aatagcacac agcattctct agtcaatatc      420
tttagtgatc ttctttaata aacttgaaag c                                                                                   451
```

<210> 40

<211> 778
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(778)
 <223> n = A,T,C or G

<400> 40
 accgcatacct agccgcccagac tcacacaagg caggtgggtg aggaaatcca gagttgccat 60
 ggagaaaatt ccagtgtcag cattcttgct ccttggtggc ctctctaca ctctggccag 120
 asataccaca gtcaaacctg gagccaaaaa ggacacaaag gactctcgac ccaaactgcc 180
 ccagaccctc tccagaggtt ggggtgacca actcatctgg actcagacat atgaagaagc 240
 tctatataaa tccaagacaa gcaacaaacc cttgatgatt attcatcact tggatgagtg 300
 cccacacagt caagctttaa agaaagtgtt tgctgaaaat aaagaaatcc agaaattggc 360
 agagcagttt gtcctcctca atctggttta tgaaacaact gacaaacacc tttctcctga 420
 tggccagtat gtcccaggga ttatgtttgt tgacctatct ctgacagtta gagccgatat 480
 cactggaaga tattcaaatc gtctctatgc ttacgaacct gcagatcagc tctgttgctt 540
 gacaacatga agaaagctct caagttgctg aagactgaat tgtaaagaaa aaaaatctcc 600
 aagcccttct gtctgtcagg ccttgagact tgaaaccaga anaagtgtga naaagactgg 660
 ctagtggggg aagcattagt ggaacacact ggattanggt tatgggtttt aatggttacc 720
 aacaacctat ttttttttaa naaaaaaacc aaagttttta aaaaaaattt nggggtttt 778

<210> 41
 <211> 696
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(696)
 <223> n = A,T,C or G

<400> 41
 agtgatgccg gcagatcacc tggtttcagc gggacnacgt atcttgcatg tggctctggca 60
 tggacnccag ggctgtgggg acttggggta cagtaatcaa gtaatcccct tttccagaat 120
 gcattaaccc actccctttg acctcacgat ggggcaggtc cccaagtgtg caagctcagt 180
 attcatgatg gtgggggatg gagtgtcttc cgaggttctt gggggaaaaa atattgtagc 240
 atatttaagg gaggcaatga accctctccc ccnctcttc cctgcccata tctgtctcct 300
 agaattctat gtgctgtgaa taataggcct tcwctgcccc tccagttttt atagacstga 360
 gggtccagtg tctcctggta actggaacct ytctgaggg ggaatcctgg tgctcaaatt 420
 accctccaaa agcaagtagc caaagccgtt gccaaacccc acccataaat caatgggccc 480
 tttatttatg mcgactttat ttattctaat atgattttat agtatttata tatattgggt 540
 cgtctgcttc ccttgatttt ttmttccttt ttttgtaata ttgaaaasgg sgatataatt 600
 attataagta gactataata tatttagtaa tatatattat taccttaaaa gtctattttt 660
 gtgttttggg cattttttaa taaacartct gagtgt 696

<210> 42
 <211> 509
 <212> DNA
 <213> Homo sapien

<400> 42
 ctcagggtga aaaggaggga gctactctca ggctgcgtgc agcgacagtg cccagggtc 60

tgatgtgtct	ctcacagctt	gaaaagcctg	agacagctgt	cttgtgaggg	actgagatgc	120
acgatttctt	cacgcctccc	ctttgtgact	tcaagagcct	ctggcatctc	tttctgcaaa	180
ggcacctgaa	tgtgtctcgc	tccctgttag	cataatgtga	ggaggtggag	agacagccca	240
cccttgtgtc	cactgtgacc	cctgttccca	tgctgacctg	tgtttccctc	ccagtcattc	300
ttcttgttcc	acaagagggtg	gggctggatg	tctccatctc	tgtctcaact	ttacgtgcac	360
tgagctgcaa	cttcttactt	ccctactgaa	aataagaatc	tgaatataaa	tttgttttct	420
caaatatttg	ctatgagagg	ttgatggatt	aattaaataa	gtcaattcct	ggaatttgga	480
gagagcaaat	aaagacctga	gaaccttcc				509

<210> 43

<211> 388

<212> DNA

<213> Homo sapien

<400> 43

gcaggatttc	ttcactctc	cccttgtga	cttcaagagc	ctctggcatc	tctttctgca	60
aaggcacctg	aatgtgtctg	cgtccctgtt	agcctaattg	gaggaggtgg	agagacagcc	120
cacccttgtg	tccactgtga	cccctgttcc	catgtgacc	tgtgtttcct	ccccagtcac	180
ctttcttgtt	ccagagaggt	ggggctggat	gtctccatct	ctgtctcaac	tttatgtgca	240
ctgagctgca	acttcttact	tccctactga	aaataagaat	ctgaatatac	atttgttttc	300
tcaaawattt	gctatgagag	gttgatggat	taattaaata	agtcaattcc	tggaatgtga	360
gagagcaaat	aaagacctga	gaaccttc				388

<210> 44

<211> 565

<212> DNA

<213> Homo sapien

<400> 44

ggaaaagagg	gttccctctc	ttgtgwtgt	ctcttcccc	cacccctaatt	tcttctgtct	60
tgtttgggaa	gacgtggagg	aaaagggtgac	ttctgcccc	accgtcttta	ccccaccaa	120
ctctcgcgtg	ttggagagaa	ggggccctcc	cagcaciaag	ttgcattcct	ccccctaat	180
ttattctaatt	ttattaactt	tgaccacccc	ttcttgagcc	tgcagccttc	ccgtgtggcc	240
tgagggctgt	cgagtgaact	gccccagccc	ctcccagccc	ttgcccagcc	tgggggagtg	300
gggaaggctt	gggcatggcc	ccgttggagg	ttgatttgc	gttttgttct	ttgtctttgt	360
gttctgtggt	acttgcctgag	agaaaagaaa	agtgaagcaa	gcagaaggag	gtgggaaaac	420
ggacccaaac	cccagtggtc	cctgccccga	tgcccttctc	ttagtgggtg	gaaaccctta	480
tctygcaaa	ggaatgtgtc	cccttcccc	cccthtagt	tatttcacag	aaaacaaaac	540
ctcccaataa	aacggttgaa	gcctg				565

<210> 45

<211> 661

<212> DNA

<213> Homo sapien

<400> 45

gttgaggaga	tgggatgtcc	cagatgatag	ggctcctggg	atttcagacc	caataccagc	60
aggactccag	tcacctctac	cccakttctc	caggacacag	cgctcccaac	tctgagtgc	120
gtcccacctc	tggtccttgc	agcacaacca	acgtgggaat	cacaccctcc	agacctccca	180
cagctccacc	ccagactggg	cgccggccct	gcctccattt	cagctgtgac	aacctcagag	240
ccgtgttggc	ccaagcatga	caaggacgta	tgaaaacttc	cagtacttgg	agaataagg	300
gaaagtccag	gggttttaaaa	atggggccact	tcctctccag	tccctcctgc	agcgtctccg	360
ctctggggcc	tgccatctcc	tgtgtccct	gggctcggc	ctgtgtgtgc	tggtcatcat	420
ctgtgtgggt	ggatttccaa	attccaaatt	tcagagggac	ctggtgaccc	tgagaacaga	480
tttagcaac	ttcacctcaa	acactgtggc	ggagatccag	gcactgactt	cccagggcag	540

```

cagcttggaa gaaacgatag catctctgaa agctgaggtg gagggtttca agcaggaacg      600
gcaggcagtt cattctgaaa tgctcctgcg agtccagcag ctggtgcaag acctgaagaa      660
a                                                                           661

```

```

<210> 46
<211> 453
<212> DNA
<213> Homo sapien

```

```

<400> 46
tgctctgtgt gtgtgcctgc tgaataactt ctatcccaga gaggccaaag tacagtggaa      60
ggtggataac gccctccaat cgggtatctc ccaggagagt gtcacagagc aggacagcaa      120
ggacagcacc tacagcctca gcagcaccct gacgctgagc aaagcagact acgagaaaaca      180
caaagtctac gcctgcgaag tcacccatca gggcctgagc tcgcccgtca caaagagctt      240
caacagggga gagtgttaga gggagaagtg ccccccacctg ctctcagtt ccagcctgac      300
cccctcccat cctttggcct ctgacccttt tccacaggg gacctacccc tattgcggtc      360
ctccagctca tctttcacct cccccctc ctctccttg gctttaatta tgctaagtgt      420
ggaggagaat gaataaataa agtgaatctt tgc                                           453

```

```

<210> 47
<211> 687
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1) ... (687)
<223> n = A,T,C or G

```

```

<400> 47
cttcggctaa aatgcagaaa tgcattgctgt cagcgttggt atttcacatt caatggagct      60
gaatgttsag gacctcttcc cattgaagct ataatttatt tggaccaagg aagccctgaa      120
atgaattcaa caattaatat tcatcgcaact tcttctgtgg aaggactttg tgaaggaatt      180
ggtgctggat tagtggatgt tgctatctgg gttggcactt gttcagatta cccaaaagga      240
gatgcttcta ctggatggaa ttcagtttct cgcattcatta ttgaagaact accaaaataa      300
atgctttaat tttcatttgc tacctctttt tttattatgc ctgggaatgg ttcacttaaa      360
tgacatttta aataagttta tgtatacatc tgaatgaaaa gcaaagctaa atatgtttac      420
agaccaaagt gtgatttcac actgttttta aatctagcat tattcatttt gtttcaatca      480
aaagtgggtt caatattttt tttagttggt tagaatactt tcttcatagt cacattctct      540
caacctataa tttggaatat tgttgtggct tttgtttttt ctcttagtat agcattttta      600
aaaaaatata aaagctacca atctttgtca atttgtaaat gttaaanaat tttttttata      660
tctggtnaaa taaaaaatta ttttccc                                           687

```

```

<210> 48
<211> 944
<212> DNA
<213> Homo sapien

```

```

<400> 48
ggcacgagga gcatggacat gaggggtcccc gctcagctcc tggggctcct gctactctgg      60
ctccgaggtg ccagatgtga catccagatg acccagcttc catcttccct gtctgcttct      120
gtaggagaca gagtcccat cacttgccgg gcaagtcaga acattaacaa cttcttaatt      180
tggtatcagc aaaaaccagg gagaccccct cagctcctga tctatcttac atctaatttg      240
caaagtgggg tcccatctag gttcagtggc agtggatctt ggacagattt cactctcacc      300
atcacgggtc tgcagcctga agattttgca acttactact gtcaacagag tcacagtacc      360

```

ccgtacacct	tcggccaggg	gaccaagctg	gagatcaaac	gaactgtggc	tgcaccatct	420
gtcttcatct	tcccgccatc	tgatgagcag	ttgaaatctg	gaactgcctc	tgttgtgtgc	480
ctgctgaata	acttctatcc	cagagaggcc	aaagtacagt	ggaagggtga	taacgccctc	540
caatcgggta	actcccagga	gagtgtcaca	gagcaggaca	gcaaggacag	cacctacagc	600
ctcagcagca	ccctgacgct	gagcaaagca	gactacgaga	aacacaaact	ctacgcctgc	660
gaagtcaccc	atcagggcct	gagctctccc	gtcacaaaga	gcttcaacag	gggagagtgt	720
tagagggaga	agtgccecca	cctgctcctc	agttccagcc	tgacccctc	ccatcctttg	780
gcctctgacc	ctttttccac	aggggacctc	cccctattgc	ggctcctcag	ctcatctttc	840
acctcacccc	cctcctcctc	cttggtctta	attatgctaa	tgttgaggga	gaatgaataa	900
ataaagtga	tctttgcacc	tgtaaaaaaa	aaaaaaaaaa	aaaa		944

<210> 49

<211> 449

<212> DNA

<213> Homo sapien

<400> 49

ggcacgaggg	cttcgaggaa	ccagccctga	tacctgtgac	cctctctgta	cgctaccctc	60
ctgaagtgtc	catctccggc	tatgatgaca	actgggtacct	cgcccgctact	gatgccaccc	120
tgagctgtga	cgteccgagc	aaccagagc	ccacgggcta	tgactggagc	acgacctcag	180
gcaccttccc	gacctccgca	gtggcccagg	gtcccagct	ggcatccac	gcagtggaca	240
gtctgttcaa	taccaccttc	gtctgcacag	tcaccaatgc	cgtgggcatg	ggccgcgctg	300
agcagggtcat	ctttgtccga	gaaaccccca	gggcctcgcc	ccgagatgtg	ggcccgctgg	360
tgtggggggc	cgtggggggg	acactgctgg	tgtgctgct	tctggctggg	gggtccttgg	420
ccttcacct	gctgagggtg	aggaggagg				449

<210> 50

<211> 703

<212> DNA

<213> Homo sapien

<400> 50

ggcacgaggg	tgagcccatg	ccccacccaa	ccctgccagc	tgagcaaagg	acagtcttac	60
agcgtcaatg	tcaccttcac	cagcaatatt	cagtctaaaa	gcagcaaggc	cgtgggtgcat	120
ggcatcctga	tgggcgtccc	agttcccttt	ctcattcctg	agcctgatgg	ttgtaagagt	180
ggaatttaact	gccctatcca	aaaagacaag	acctatagct	acctgaataa	actaccagtg	240
aaaagcgaat	atccctctat	aaaactgggtg	gtggagtggc	aacttcagga	tgacaaaaac	300
caaagtctct	tctgctggga	aatcccagta	cagatcggtt	ctcatctcta	agtgcctcat	360
tgagttcggg	gcactctggc	aatgagtctg	ctgagactct	tgacagcacc	tccagctctg	420
ctgcttcaac	aacagtgact	tgtcttccaa	tggtatccag	tgattcgttg	aagaggagggt	480
gctctgtagc	agaaactgag	ctccgggtgg	ctggttctca	gtggttgtct	catgtctctt	540
tttctgtctt	aggtgggttc	attaaatgca	gcacttgggt	agcagatgtt	taattttttt	600
tttaacaaca	ttaacttggtg	gcctctttct	acacctggaa	atttactctt	gaataaataa	660
aaactcggtt	gtcttgtcaa	aaaaaaaaaa	aaaaaaaaaa	aaa		703

<210> 51

<211> 963

<212> DNA

<213> Homo sapien

<400> 51

caatgaggct	ccctgctcag	ctcctggggc	tgtaatgct	ctgggtctct	ggatccagtg	60
gggatattgt	gatgactcag	tctccactct	cccagcccg	cacccctgga	gagccggcct	120
ccatctcctg	caagtctagt	cagagcctcc	tacatagtaa	tggatacagt	tatttggatt	180
ggtaacctgca	gaagccagga	cagctctccac	agctcctgat	ctatttggct	tctaagcggg	240

```

cctccgggggt ccttgacagg ttcagtggca gtggatcagg cacagatttt acactgagaa 300
tcagtagagt ggaggctgag gatgttggct tttattactg catgcaaact ctacagacct 360
cgctcacttt cggcggaggg accaagggtg agatcaaacg aactgtggct gcaccatctg 420
tcttcatctt cccgccatct gatgagcagt tgaaatctgg aactgcctct gttgtgtgcc 480
tgctgaataa cttctatccc agagaggcca aagtacagtg gaagggtgat aacgcctccc 540
aatcgggtaa ctcccaggag agtgtcacag agcaggacag caaggacagc acctacagcc 600
tcagcagcac cctgacgctg agcaaagcag actacgagaa acacaaactc tacgcctgcg 660
aagtcaccca tcagggcctg agctcgcccc tcacaaagag cttcaacagg ggagagtgtt 720
agagggagaa gtcccccccc ctgctcctca gttccagcct gacccccctc catcctttgg 780
cctctgaccc tttttccaca ggggacctac ccctattggg gtccctccagb tcwtttttcc 840
cctccccccc ctccctcctc ttggbtttaa ttatgytaak gttggrggar aatgaataaa 900
taaagtgawt ttttgmaaaa aaaaaaaaaa aaaaaaaaaa aaaaagaaaa aaaaaaaaaa 960
aaa

```

<210> 52

<211> 628

<212> DNA

<213> Homo sapien

<400> 52

```

cctttctctc tctcatttc ggtgcatgtc ctttctgcag ctgcctttca gcacagggtg 60
ttccactggg ggcagctaac gctgagtgaac aaggatggga agccacaggt gcattttact 120
caagtcttct ctagtcaatg aggggcaccc agtgcttctg gggcaggctg ggtgggtggc 180
ccctaggtat cagcctctct tactgtactc tccgggaatg ttaacctttc tattttcagc 240
ctgtgccacc tgtctaggca agctggcttc ccctattggc cctgtgggtc cacagcagcg 300
tggtgcccc ccagggccac cgcttctttc ttgatcctct tctcttaaca gtgacttggg 360
cttgagtctg gcaaggaacc ttgcttttag cttcaccacc aaggagagag gttgacatga 420
cctccccgc cctcaccacaa ggctgggaac agaggggatg tggtagagc caggttcctc 480
tgccctctc caggggtgtt tccactagtc actactgtct tctccttgta gctaataaat 540
caatattctt cccttgctg tgggcagtgg agagtgtctc tgggtgtacg ctgcacctgc 600
ccactgagtt ggggaaagag gataatca
628

```

<210> 53

<211> 598

<212> DNA

<213> Homo sapien

<400> 53

```

ctccctccct ttttccacc ctccacctct caggggccct gccccagccc ctaagccccg 60
tcccctaacc cccagtctc actgtcctgt tttcttaggc actgagggat ctgagtaggt 120
ctgggatgga caggaaaggg caaagtaggg cgtgtggttt cctgccccct gtccggaccg 180
ccgatcccaa gtgcgtgtgt ctctgtctct cctagcccc ytytcacaca tcacattccc 240
atggtggcct caaaaaaggc ccggaagcgc caggctggag ataacagcct yttgcccgtc 300
ggccttgctg cgccctggg gtaccatgtg gccacaactg ctgtggcccc ctgtcccaa 360
racacttccc cttgtctccc tggttgcty tcttgccct tgtcctgaag cccagcgaca 420
caraaggggg tggggcgggt ytattgggar aaaggagcg aggtcaragg agggcatggg 480
ttggcagggt gggcgtttgg ggccttytat gctggctttt caccctcarag gacacaggca 540
gcttccaaaa wawatttaty ttcttcacgg gaaaaaaaaa aaaaaaaaaa aaaaaaaa 598

```

<210> 54

<211> 592

<212> DNA

<213> Homo sapien

<400> 54


```

gtgttttacac agtaagatca agagtcaggc cctggaattt ccagaaccag cccgacatag      60
ttgaggactt gaaggacctg atcaccctgt atgctggaca agaaccctga gtcgaggatc      120
gtggtgccgk aaatcaagat mctggtraag accatratac gtaaaccgtc ctttgggaac      180
ccattcgagg gcascggmg ggaggaacgc tcaactgtcag ygcctggaaa cttgctcacr      240
aagcaaggca gygaagacaa cctccagggc wccgaccgc ccccggtggg ggaggaggaa      300
gtgctmttgt gagaggcagt ccctgctgtg aaagtgtgtg ggccccagc ccccggtccc      360
ccgcacgca tgcattccact gcggccggag gaggccatgg agcccgagta gctgcctgga      420
tcgctcgacc tcgcatcgcc gcgcgctcgc ctctgggggg atgctgcacc gcgtttccat      480
agcagcatgt cctacggaaa cccagcacgt gtgtagagcc tcgatcgtca tctgctggtt      540
atttgttttt tcctttgttg ttttaaaggg gacaaaaaaa aaaaaaaaaa aa          592

```

```

<210> 55
<211> 504
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(504)
<223> n = A,T,C or G

```

```

<400> 55
agcgggacga ggcckngygg acttatcggg gcccggggac cgggggcgcg cggaggggtgc      60
acttcaccct cctgcccgak cgctacgagc cactggagga gccggcgccg agcgagcagc      120
ccaggaagag gtaccggagg aagctgaaga agtactggca agaattgtcg gaaggtcatc      180
atmaaakgat gccgtactcg ggtcatcgcc ctgcaaggmt tcgctgcags ctactccgcc      240
ccgtttgcgg tagccaccag cgtgggtatct ttcgtgcgct aatgggagct gctgtgycag      300
gtgccccag agtgaacggg agccccgtct gtgggaactt tgtgaatcct ggagcatctc      360
atacttgaac acacakcata tttggaagag aaaacatgcc ttcttttgnr gaatcacatt      420
agtatgatga gtgagtcatn cctgcccata tgctgagctt ctacatctc tcagtcacac      480
tgggaccacg tgggtcaatcc tgca          504

```

```

<210> 56
<211> 749
<212> DNA
<213> Homo sapien

```

```

<400> 56
gtaagataca agtttcaggc aggggtctgaa ggactggatt ggccaaacat cagacctgtc      60
ttccaaggag accaagtcct ggctacatcc cagcctgtgg ttacagtgcg gacaggccat      120
gtgagccacc gctgccagca cagagcgctc ttccccctgt agactagtgc cgtagggagt      180
acctgctgcc ccagctgact gtggccccct ccgtgatcca tccatctcca gggagcaaga      240
cagagacgca ggaatggaaa gcggagtcc taacaggatg aaagtcccc catcagttcc      300
cccagtcct ccaagcaagt agctttccac atttgtcaca gaaatcagag gagagatggt      360
gttgggagcc ctttgagaaa cgccagtctc ccaggcccc tgcattctatc gagtttgcaa      420
tgtcacaacc tctctgatct tgtgctcagc atgattcttt aatagaagtt ttattttttc      480
gtgcactctg ctaatcatgt gggtgagcca gtggaacagc gggagacctg tgctagtttt      540
acagattgcc tctaatgac gcggctcaaa aggaaaccaa gtgttcagga gttgtttctg      600
accactgat ctctactacc acaaggaaaa tagtttagga gaaaccagct tttactgttt      660
ttgaaaaatt acagcttcac cctgtcaagt taacaaggaa tgctgtgccc aataaaagg      720
ttctccaaaa aaaaaaaaaa aaaaaaaaaa          749

```

```

<210> 57
<211> 673
<212> DNA

```

<213> Homo sapien

<400> 57

agatgtcatg	acccatcaac	gtctcctctc	attgtccctc	tgctgggaca	aagtgccttg	60
tgtctggctg	ggggacaacc	aagagccccc	aagtgcactt	ccctaaggte	ctccagtgtc	120
tgaacatcag	cgtgctaagt	cagaaaaggt	gcgaggatgc	ttacccgaga	cagatagatg	180
acaccatgtt	ctgcgccggg	gacaaagcag	gtagagactc	ctgccagggt	gattctgggg	240
ggcctgtggg	ctgcaatggc	tccctgcagg	gactcgtgtc	ctggggagat	tacccttgtg	300
cccggcccaa	cagaccgggt	gtctacacga	acctctgcaa	gttcaccaag	tggatccagg	360
aaaccatcca	ggccaactcc	tgagtcattc	caggactcag	cacaccggca	tccccacctg	420
ctgcagggac	agccctgaca	ctcctttcag	accctcattc	cttcccagag	atgttgagaa	480
tgttcattct	tccagccctc	gaccccatgt	ctcctggact	cagggtctgc	ttccccaca	540
ttgggctgac	cgtgtctctc	tagttgaacc	ctgggaacaa	tttccaaaac	tgtccagggc	600
gggggttgcg	tctcaatctc	cctggggcac	tttcatctc	aagctcaggg	cccatccctt	660
ctctgcagct	ctg					673

<210> 58

<211> 994

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(994)

<223> n = A,T,C or G

<400> 58

gacgggccc	gtgctgaagg	gcagggaaca	acttgatggt	gtacttttga	actgcttttc	60
ttttctcctt	tttgacaaa	gagtctcatg	tctgatattt	agacatgatg	agctttgtgc	120
aaaaggggag	ctggctactt	ctcgtctctg	ttcatccac	tattattttg	gcacaacagg	180
aagctgttga	aggaggatgt	tcccatcttg	gtcagtccta	tgcggataga	gatgtctgga	240
agccagaacc	atgccaaata	tgtgtctgtg	actcaggatc	cgttctctgc	gatgacataa	300
tatgtgacga	tcaagaatta	gactgcccc	acccagaaat	tccatttggg	gaatgttgtg	360
cagtttgccc	acagcctcca	actgctccta	ctcgccctcc	taatggtcaa	ggacctcaag	420
gccccaaagg	agatccaggc	cctcctggta	ttcctgggag	aaatggtgac	cctggtattc	480
caggacaacc	agggtccctt	ggttctcctg	gccccctgg	aatctgtgaa	tcatgcccta	540
ctggtcctca	gaactattct	cccagtatg	attcatatga	tgtcaagtct	ggagtagcag	600
taggaggact	cgcaggetat	cctggaccag	ctggccccc	aggccctccc	ggtccccctg	660
gtacatctgg	tcatectggt	tccccctggat	ctccaggata	ccaaggaccc	cctggtgaac	720
ctgggcaagc	tggtccttca	ggccctccag	gacctcctgg	tgctataggt	ccatctggtc	780
ctgctggaaa	agatggagaa	tcaggtagac	ccggacgacc	tggagagcga	ggattgcctg	840
gacctccagg	tatcaaaggt	ccagctggga	tacctggatt	ccctgggatg	aaaggacaca	900
gaggcttcga	tggacgaaat	ggagaaaagg	gtgaaacagg	tgtcctcgga	ttaaagggtg	960
aaaaggtctt	ccaggcgaaa	aggagcnaca	agtt			994

<210> 59

<211> 639

<212> DNA

<213> Homo sapien

<400> 59

tgggtggtgg	ggaggacctg	cactctggcg	cccagcagca	ctggtcgggc	cactctgcgg	60
agatctccac	gctggccctc	agccacagtg	cccaggctct	ggcctctgcc	tggggccgaa	120
gcagcacgac	cgccatttgt	cagatccggg	tctgggacgt	gtctggcggc	ctctgccagc	180
atctcatttt	ccccatagc	accaccgtgc	tggccctggc	cttctcacca	gatgacaggc	240

```

ttcttgtcac actgggggac cacgatggcc gcacctcgc cctgtggggc acggccacct 300
atgacctcgt gtcctccacc cgcctcccgg agccgggtgca tgggtgtggcc ttcaaccctt 360
gggacgcggg cgagctcacc tgtgtggggc agggcactgt caccttctgg ctcttcagc 420
agcgtggggc agacatcagc cttcaggtgc gtcgagagcc agtcccagag gcagtggggg 480
ctggagagct gacctcgctc tgctacgggg cacctccctt gctctattgt ggcaccagct 540
ctggccaggt ctgtgtctgg gacacgcgtg ccggccgctg cttcttgtcc tgggaggcgg 600
atgacggtgg cattgggctg ttgctgttct cgggttctc 639

```

<210> 60

<211> 470

<212> DNA

<213> Homo sapien

<400> 60

```

ggcacgaggt ttttattctt tgcacaattg tttcattgtt tgacacttaa tgcactcgtc 60
atttgcatac gacagtagca ttctgaccac acttgtacgc tgtaacctca tctacttctg 120
atgtttttaa aaaatgactt ttaacaagga gagggaaaag aaacccta aattttgctt 180
tgtttccttg aagaatgtgg caacactgtt ttgtgatttt atttgtgcag gtcattgcaca 240
cagttttgat aaagggcagt aacaagtatt ggggcctatt tttttttttt tccacaaggc 300
attctctaaa gctatgtgaa attttctctg cacctctgta cagagaatac acctgccctt 360
gtatatcctt ttttccctc cctccctcc cagtgggtact tctactaaat tgttgtcttg 420
ttttttattt tttaataaaa ctgacaaatg aaaaaaaaaa aaaaaaaaaa 470

```

<210> 61

<211> 535

<212> DNA

<213> Homo sapien

<400> 61

```

ggcacgaggg gttcttgggc caatttcttg ctgactcagc cccactctgt gtcggagtct 60
ccggggaaga cggtaaccat ctctgcacc ggcaacagag gcagcattgc cgccaactat 120
gtgcagtggg accagcagcg cccgggcagt gccccacca ctgtgatcta tgaagataac 180
caaagacctt ctgggggtccc tgatcgggtc tctggctcca tcgacagctc ctccaactca 240
gcctccctca ctatctctgg actgaagcct gaggacgagg ctgactacta ctgtcagtct 300
tatgagagca gcaatcattg ggtgttcggc ggagggacca agctgaccgt cctaggtcag 360
cccaaggctg cccctcgggt cactctgttc ccaccctct ctgaggagct tcaagccaac 420
aaggccacac tgggtgtgtc cataagtgc ttctaccgg gagccgtgac agtggcctgg 480
aaggcagata gcagccccgt caaggcggga gtggagacca ccacaccctc caaac 535

```

<210> 62

<211> 696

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(696)

<223> n = A,T,C or G

<400> 62

```

gccagtatcc aatggggaat cagctctaaa aggaaccaga ccaacgtttt ccagcccntt 60
cattntgggt actgagggga ggaagaatg ggagggggta ttcttgtcta gtggatggaa 120
aggaaacaca ctgtcaaatt actatatctc cttggttttc tattacagta gaattctcca 180
gccatatttt tattgtctat gggggaagtt ggagatggtg accttgatta gaagtgtctg 240
gagggggata aatggagggg ataagattca gttggttttg gaaaatgtta aagtcttaaa 300

```

```

ataatgcgtc catctgaaga attttttcta aaaccagagt ttataaaaat atcactgata 360
cagcctgccc cctcatttcc ctgccacagg agatgtcttg gactagagac acttgtttaa 420
taatagcttg tctctgatat tcccagtagc ttccctctgt gtgaggaaag gatagaaatg 480
ttcaggacat catcatacag gctcctcatc tacaaagttc cagtagcagt gacgcctaca 540
cggaagactt ggaactgcaa acaggctggg gtcacctcag tgacatctga cgctgtccaa 600
ccagaagttc gatttttgtt ctgggggtga aggaggaaac agacgtgtac taaaggacta 660
aaataatttg tctatacaaa aaaaaaaaaa aaaaaa 696

```

```

<210> 63
<211> 256
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(256)
<223> n = A,T,C or G

```

```

<400> 63
taatatttat tgaagnccta ctttgctctc tacatagggg agcttctgtc agggaatgtt 60
ggttcttccc aagaaacact gattttcttt cagggagact tcatgtgttc atttatttcc 120
accacagcag attttaagaa attataatat gtaatatattg atatctataa agagtanatc 180
taacgtgaat aaattatgaa gcatactaata gagtacctat gacccatnac acatatacat 240
taaaacattt taaata 256

```

```

<210> 64
<211> 678
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(678)
<223> n = A,T,C or G

```

```

<400> 64
ggcacgaggg ctanaagcca tcgcatcttt ctccgtattc aacaagccca ttataacaag 60
tctttcacgc tcaaccattc aaacaatccc aacaatccca gcctaagaat ttcttgccat 120
ttgatgaaaa tggctgtggg ttttgctatc tttaagctcc ctgggaagca ggatataagc 180
ccagggctgg caggctcttt cttagctacct gctcttgcac atagagtttg cctcatggtt 240
gcaagaaggc tgccatagct ccagatggca catagacatt ccaggcagca ggaaaaagga 300
aggagcactt aaccgaagga ggtcaggag ttggttagtc tccacctgaa gaagagagcc 360
atgaaccagc ttcagttgac taacgggctc ctgtgagtgc atctttggac ttttctggag 420
gttgaaatct agatgtggta tgtgtcttaa agcagccacc aactcctccc attaccttcc 480
aagtgaagccc aactacacga tggagcctct tccctgccct tggatctggg ctgagcctct 540
gacttgcggt gaccaacaga atgcagtga agtgatgccg atactaccct ccctgcccta 600
gacttgggat acctgcagct atattcttcc attcctcagg acttgcaaaa acgggtctca 660
ncatgccttn ccagagcc 678

```

```

<210> 65
<211> 678
<212> DNA
<213> Homo sapien

```

```

<220>

```

<221> misc_feature
 <222> (1)...(678)
 <223> n = A,T,C or G

<400> 65
 ggacagagga ggacacactc aggatagaaa ctttatgtgt atagggaaatg tggtagagcc 60
 tttagccagg agtcatactt catcagacac cagaggacac acacagtgtg gtggcttttt 120
 cagccattgc tagataccaa agtggagaca ttctgtgtgt gattatgcat gagactgtac 180
 tggtaagact tgtatctcca tccacctgaa ggagaattgc tggctcattt tcaggagccc 240
 tgcccttccct cactgtggat ggtgggttgt ggaaacccgg tcaggtaatg atagtggcag 300
 gaggcagtca aatgcccagg cagatagggg tgggtacctg gtgaaacca accttaaagc 360
 tgaagacagt cccggctaaa tctcatact gaattgagaa cctgtcttcc catttggtgt 420
 gctttccctcc gattgatccc aacccttcac ctattttacg tataacctgcc ctttcctaata 480
 tggttttttac actgctgtgc ccaccttttg agtgggtgct ttgcatactt acaaatacagt 540
 caacgtgtat tccccatttc tgagcccata aaagaccan actcagctgc agtgaggaga 600
 gaaatcccct gctgnggggg gtggggacca ctccctgcat ccctctncac tganagctgt 660
 ctttttgctc aataaaat 678

<210> 66
 <211> 606
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(606)
 <223> n = A,T,C or G

<400> 66
 gcatcaactg ccgccagccc ttcattntnt ccgcctcttc ctagacgtgc tacacctggt 60
 tgagttctac ctggaggaag ggatcactga tgaagaagcc atctccctca tcgacctgga 120
 ggtgctgaga cccaagcggg atgacagaca gctagagatt gcaaacaaca gctcccagat 180
 tctgcggcta gtggagacca aggactccat cggagatgag gacccttca cagctaagct 240
 gagctttgag caaggtggct cagagttcgt gccagtgtg gtgagccggc tgggtgctgcg 300
 ctccatgagc cgcgggatg tctcatcaa gcgatggccc ccacctga ggtggcaata 360
 ctccgctca ctgctgctg acgcctccat taccatgtgc ccctcctgct tccagatgtt 420
 ccattntgag gactatgagt tgctgggtgct tcagcatggc tgctgcccct actgccgag 480
 gtgcaaggat gacctgggc catgaccagc atcctgggga cggcctgcac cctctgcccg 540
 ccttgggggtc tgctgggctg tgaaggagaa taaagagtta aactgtcaaa aaaaaaaaaa 600
 aaaaaa 606

<210> 67
 <211> 579
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(579)
 <223> n = A,T,C or G

<400> 67
 ggcacnaggc ccaattatat gtccattttg tatatttttt ggtcggggga aaaattgacc 60
 tgcagtanaa aaacctttga ccatttttat gtccattgga tactttcctt tttatcatct 120
 taaaaaaga taactagtac taatcattgt agtggcctaa gtgtgattta actcttgaag 180

```

tcacaccctc cgaaagatga gtagaaacca gcaccagcac agcccagatc ttctctttcc 240
tctccttttc ctcatttatt cctaaaggaa tctgaccatt ttacgtctct acggcccaaa 300
aaaaagacaaa aataaaaatt cctttttatt cctgtcaact ggatggaaac acaaatttca 360
tggagctgtg taccatcgaa gaaacctggg gtctggcatg aaattactgt aaagaacttc 420
ctgtaaaaca cgttctttaa caaactgaaa tgaaaagcat tggagcgtct gaatgaaaga 480
cgtgacctcc tgctgggact ctgatggtct tcagcattca ccttcgtgtg tcttcagtgt 540
ctcattgtca tccctgcttc tgtttgggtct tagagtgtt 579

```

```

<210> 68
<211> 258
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(258)
<223> n = A,T,C or G

```

```

<400> 68
ggcatntggc cctgaccana tccttaaccc tagcgatggc ttgggagatg ggggggttggg 60
tagcattntc tttcttggcc ctcccttatt ctaggaaaa atggttccct tccttgtgtg 120
tgtctcttcc ccccccctt aattcttctg ctctgtttgg gaagacgtgg aggaaaagg 180
gacttctgcc cccaccgtc ttacccccac tgtantggcc tttggagatg ccccccctc 240
ccccccacca actctcgc 258

```

```

<210> 69
<211> 628
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(628)
<223> n = A,T,C or G

```

```

<400> 69
ggcacnagga gaattnttg gaataaaact gactgataaa actggaaatg ttgctcgggc 60
tgtaactgg caggaaagat ttcagcatct gaatgagtc cngcacaact atttaagaat 120
cactcgtatt cttaaaagcc ttggtgagct tggatatgaa agtttttaat ctctcttgt 180
aaaatttatt ctcatgaag ctcttgtgga gaatactatt cccaatatta agcagagtgc 240
tctagagtat ttgttttata caattagaga cagaagagaa aggagaaagc tcctgcggtt 300
cgcccagaaa cactacacgc cttcagagaa ctttatctgg ggaccgcctc gaaaagaaca 360
gtcggagggg agcaaagccc agaaaatgtc ttccctctc gcctccagtc ataacagtca 420
aacttctatg cacaaaaaag ccaaggactc caaaaattcc tcctcagctg ttcattttaa 480
tagcaaaaca gctgaagaca aaaaagtggc accaaaagag cctgtggaag agacagacag 540
gccccccan agcccagcaa tgaagctgcc aagccaagaa atacagaaga aggacagtaa 600
tgctganaac atgaattctc aacctgag 628

```

```

<210> 70
<211> 439
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature

```

<222> (1) ... (439)

<223> n = A,T,C or G

<400> 70

```

ggcacgaggc gcacacccgc tgggcggccc ctgtggagac cctggaaaac atcatcgcca      60
ctgtagacac gaggtgcct gagttctcag agctgcaggg ctgtttccgg gaggagctca      120
tggaggcctt gcacctgcac ctggtgaagg agtacatcat ccaactcagc aaggggcgcc      180
tggtcctcaa gacggccgag cagcagcagc agctggctgg gtacatcctg gccaatgctg      240
acaccatcca gcacttctgc acccagcacg gctccccggc gacctggctg cagcctgctc      300
tccctacgct ggccgagatc attcgctgc aggaccccag tgccatcaag attgagggtg      360
ccacttatgc cacttgctac cctgacttca gcaaaggcca cctgancgct atcctggcca      420
tcaaggggaa cctatccaa

```

<210> 71

<211> 328

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1) ... (328)

<223> n = A,T,C or G

<400> 71

```

tnggaactca ggtttacnca aactgagggg gccccagccc tngtaccncc cctgttacc      60
caggatccat ntgcctcan aaaagngttc aggtacagca gctgaggctg ccctgaggaa      120
tcaaggggcc attaccaagg ggcaggaaaa ggatatgtaa naggnggcct tcatggtana      180
gcttgaccca anaactactc cncattngga tggcccagac tgactccatc ccctgacttt      240
ccctttgact tcncctgtt tgtaataaaa acaataaaat ggaagggtgt gtggacggga      300
aaaaaaaaa aaaaaaaaaa aaaaaaaa

```

<210> 72

<211> 721

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1) ... (721)

<223> n = A,T,C or G

<400> 72

```

ggtttctggt gctattctgc caagttatcg agtcctcct catgtttcaa cattccatct      60
tcccgtttct atcctcgact ccaaagtaag cttcttagc tccaatcagg gatgaggggc      120
tcaacctctt ctgtcctcaa agaggccaaa cgcagtgcc aagtcggtag ccttcacttt      180
tagatgtcct attcatgtaa aaaagaaggt gccccacca ggcttacatc agcaataagc      240
aattctaag caacgatggg gtccacatct taccacagtg tgtgcccag tatgcctttg      300
tgcccgtgta attattgtta ggcgcccttt cacttagagg ggtgatgata aactgtggcc      360
accttgatta caaccacat ttcttgcctt ggggagcttc caagtaacag gccatttctt      420
acctccctcc aggaacagtg ggcactgccc accacctcgt gtctgctcat aggatgacgc      480
tggagatccc cacacttact ctacctctt ggcaaattgg cattccgggtg gtggtttttg      540
tttcttttaa cacattaaat aaatgagtat ataggatgtg aggggagggg tgagaacaac      600
tagctgtagc atgtgtagc tatatacttt accattcgac ttcttttctt tttttttttt      660
aaaaaaaaa aagtgcctga ctgggttcaa gcttcatcat gaaaaaaaaa aaaaaaaaaa      720
a

```

<210> 73
 <211> 596
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(596)
 <223> n = A,T,C or G

<400> 73
 attgtacaga acagcccagg tagggaggac agntgcccga ggtcccatag gantgcatgc 60
 ctcaagccca cgtcatgcag agccactcag ctcaccctgn tcagggcacg tggtttacct 120
 gcattccccct nttgcaggtc ctacgtgtgg aggacgtacc atntgacctn tgcaggagag 180
 aaactcacgg aagacagaaa gaagctccga gactacggca tccggaatcg anacgagggt 240
 tccttcatca aaaagctgag gcaaaaagtga gcctccagac aggacaaccc tnttcatcac 300
 tgggtggctga gctttttccc agcaggaatg ggtcctcgaa tcatcgtgcc tntttcacan 360
 aaaggacgtt gtgggtggcct caccacaggc atgcccaca gtaactgtca gcataaacct 420
 gggggccctc aggactagga cagggtgagc cagtgtctcc tcctttcatg tacttggcct 480
 gagactgacc tctccctagg tccaaatgcc ctagtccatc ggcagaccca cggcctggcc 540
 cactgtataa aataaacctg tttgcttntt agtttgaaaa aaaaaaaaaa aaaaaa 596

<210> 74
 <211> 302
 <212> DNA
 <213> Homo sapien

<400> 74
 ggcacgaggc ttaaaccaag tttctctgca gctctttcgg ttctgcttac agtgtgtggg 60
 aaatctgatt tttttccctt agtaatagtt tgataagaaa tttagtgtat tgactgcctc 120
 agtgacacaa tttatcttta aagggtgtga agctgggtgg gaccaaagt taccgtgtgt 180
 tttgtgttg attgctatct tcagaagcaa accatgtttt tcacttacag taggagtcaa 240
 caaatttggg attttagaag ggggaggagg gagctatttg tgtaagactg ctgtcatatt 300
 tg 302

<210> 75
 <211> 635
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(635)
 <223> n = A,T,C or G

<400> 75
 ggcacgaggc ttaatgtgac tttcctgagt taacactgaa tagtatttct aaaatTTTTT 60
 gtactttatt ttttaagtga acttgttcta tctatctata tatatatttg atagtttgtg 120
 gaataatata cccagctatt tccatatta aatgctaatt atcttttgat ttctttttca 180
 taagcagatc tggcatttat tacagggtcg ccgcttaaga gaactcatta taatgaacgt 240
 ttattatatt ttgcagttcc atgcctgttg tccattgatt gacatgagca cccctgtttt 300
 ctctggagaa atacctcccc tctctggggt gcttcctgtg gtagtgtctt tcaggatatc 360
 gttccactag ctacagggtga gcattttacc cattgttgga taatggtaat ctctttttca 420
 gaattttgag tctgtaattc attgtacat gaaccagaaa atgtgggaac tcattcattc 480

ttgtcccaga attctgttga gaacatccat tcattctggc taattgatta caagaataac	540
tgnggatacg atccctttan aacctgttc tctgatctgn gtgtttcttc acttctcaat	600
aaaaatgtct tttgctaaaa aaaaaaaaaa aaaaa	635

<210> 76
 <211> 678
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1) ... (678)
 <223> n = A,T,C or G

<400> 76	
ggcagcagggc tcttgattct tggcttgccct ctctctccaat tccaaactta gtgaaatggc	60
cttaagcatt ttaaactgta tgtatacatt agcgcattca tgcctttcta aacgcatttc	120
aaatgtcaac caggaaggca caccactgta ttagttttat actgccgctg taaaatttac	180
cacaaactta gtgacttaac acaaatttat tgcaattctg taggctggaa gtctgactat	240
gggtctcact ggactagaat caaggctggc aggcctgcct ccttcctgga ggttctaggg	300
gagactctgt ctctgtctcc ttcaggctgc tggcagaatc cacatccttt cgggtggcagg	360
gccaaaggccc ccactttctt gctgactgta aactaaggcc acttccagct tgtagaggct	420
gctacattc cttggctctt gggccctcc tccatcttca gagctagcag gttcagtctg	480
tgtcacgaac catttctctg gttccctgca gacaggaaag gttgtcccta aggactcatg	540
agattagggt gggcccagcc agataatata tgataatctc cctcctcaag gnttttaata	600
ttaaacacat ctgcaggaca cattttgccca tgtaactaac attcactggg ccaggggatt	660
aaggaatgaa ccctcttt	678

<210> 77
 <211> 669
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1) ... (669)
 <223> n = A,T,C or G

<400> 77	
ggcagcaggg agaactctta aaaaaaaaaa acgtttctca ctgtcttaaa tagaattttt	60
aaatagtata tattcagtgg cattttggag aacaaagtga atttacttcg acttcttaaa	120
tttttgtaaa agactataag tttagacatc tttctcattc aaatttaaag atatctttct	180
cctcttgatc aatctatcaa tattgataga agtcacacta gtatatacca ttaatacat	240
ttacactttc ttatttaaga agatattgaa tgcaaaataa ttgacatata gaactttaca	300
aacatatgtc caaggactct aaattgagac tcttccacat gtacaatctc atcatcctga	360
agcctataat gaagaaaaag atctagaaac tgagttgtgg agctgactct aatcaaagt	420
gatgattgga attagaccat ttggcctttg aactttcata ggaaaaatga cccaacattt	480
cttagcatga gctacatcat ctctagaagc tgggatggac ttactattct tgggttatatt	540
ttanatactg aanggnctga tgcttctgtt attattccaa gactggagat aggcagggt	600
aaaaagggat tattattttc ctttaatgat ggggctaaaa ttcttcttat aaaattcctt	660
aaaataagg	669

<210> 78
 <211> 134
 <212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(134)

<223> n = A,T,C or G

<400> 78

ggcacgaggg	gtcgatttta	atagcgaatc	ctttttcttg	tagaggtaag	taaaatcttc	60
ctgacaaggt	tgtcctctnt	tcacggcaca	gacaatgggc	ggnctgttta	tgaggggtga	120
gaagngacnc	ccgc					134

<210> 79

<211> 396

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(396)

<223> n = A,T,C or G

<400> 79

tanatgcaag	tntnttgtng	gatatacgta	ttgagatatt	acncctagtc	tgtggcttga	60
ctgtttcttt	tatgtctttt	gatgaatana	agttttaaat	tttgacaagg	tcaaatttat	120
ttttttcttt	tgtttgatat	tttttntctc	caatttaacc	ccaagatttc	anatattntg	180
ctctattana	taaactttat	atttttatat	ttgtgatctn	ccttgaattg	atatgtatgt	240
tgtgaattat	ggatcagggg	tntttttttc	ccccatacaa	gtatccagtc	attgtaacnc	300
tgtttattga	aanaattatc	ctttcctcat	taaattncct	tgccaattag	taaaaaatca	360
attacccata	aaaaaaaaaa	aaaaaaaaaa	aaaaaa			396

<210> 80

<211> 731

<212> DNA

<213> Homo sapien

<400> 80

tctacatcat	cctgagagcg	ccttcagact	ggacagaact	gtaggaactg	ccgtctctta	60
tggtagatgt	ggcccatcac	aatataacctg	caggttttaa	ctctggacac	ggcttattgc	120
agctggagga	gtccagggct	aacatgtgga	ctcggaagaa	gaagccgctc	ggggtgaggt	180
acaggcggtt	catcttgtag	agcccgctcc	gatccaccag	cagggtcacc	agccggatgc	240
ccgcgcccag	cacgtccaca	tctgtcacga	tcccgttcac	cgcgaattcg	ctctcgaga	300
acgcctcgcg	tctgtccaga	tcggaaccggc	aggcgcgcg	gcagggctca	gcgcggggct	360
ccgcgtccct	ctgcggcggg	ctgaggccga	agctgaggct	gaagcgcggc	ggcggcgcg	420
gcgcgggtcc	cggggccagc	gcggcgggcg	gcggccggtt	ggggtgggcg	tggccggcgg	480
agccctcggt	ggcgagcgcg	ggcgggcggg	cggcggggaa	gcgcagggcc	cgtgagcgcg	540
cgcgtcgcg	ggcgctctcc	gacgcgggct	cgcggcgcg	cgacgcggcg	ttctcggtct	600
gcgccaggcg	cgggcccggc	gggccagcgg	gcgcgcgtgc	ggcctcccgg	aagctggcct	660
ggttgteggc	cggcggtggg	ggcttcggcg	ggcgcgggtc	aggccaggcg	cggacggcg	720
gagcgacctc	g					731

<210> 81

<211> 396

<212> DNA

<213> Homo sapien

<220>
 <221> misc_feature
 <222> (1) ... (396)
 <223> n = A,T,C or G

<400> 81
 cccttcatcc caatccntga gaagttcttt ttctgcttcc tgcccaacat natcaacagg 60
 acctatttcc cattttcttg ntcttncntn anccagntat tggngngngt tttgaaatgg 120
 ctnatnatga ggaanagtnt gatccnacac ttggagganc naggggtgca ggtggtnntt 180
 tgggtgcctta atgaanagtc ggattttgaa gcagccttna gcgtgggagc cacnggcgtc 240
 ataacggatt atcccnagc cctnnggcan tacctggaca accatggnc agctgcccgg 300
 acctcctaag tccagaance tngaggtntc ctgtttntnt tccatgaaaa ataaatattt 360
 gcctttcgat caaaaaaaaa aaaaaaaaaa aaaaaa 396

<210> 82
 <211> 502
 <212> DNA
 <213> Homo sapien

<400> 82
 ctcagaggaa agaagaaaaag ggccaggagt cagacgtcac acccgggggc tcccccttcc 60
 catgtagaag tgttggcatg cgtcagtcct ctttacagag gggtaggatgt atccgtggag 120
 gaggggcctt ctctctttct aattgcacta tacttgctct agcttcagtc tggagatact 180
 taagacctcc atgggggtcgt gatccataga cttagcaagt cttgccttat ctatggagct 240
 cgatggtgag aattgtgacc attgtctgat gtccatagtt ccttccccct agattgtttc 300
 tttccacgga ttgtgttcat ctgaaccatt ttatttttta ttaccaaaag tactgtactt 360
 ggctatttgc agtggtttca aaaccaaagtg tttctttttt tgtgttttta atcttcgata 420
 cttggtgcaa tagaagctgc aaagatgtgc cactttatct atgaaatgga gttttgtata 480
 ccaataaatt ctagttaaaa aa 502

<210> 83
 <211> 666
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1) ... (666)
 <223> n = A,T,C or G

<400> 83
 gatttttggg atttgactt atttatgaac ttactttgag acagaatatg gttaaaatta 60
 ggaacatcta ctttgaatga ggtttatatt tctattttga atttgcctta tgtatattca 120
 aaggcttatg gaaatactgt aaaggaacat taggaaaagg acaaataggc tataaccatc 180
 tatcttaaaa tcagaccctt agtataagca cctctttttc ttttcctctt tgacaattta 240
 gtctcttatt taggtccatg taattaatatt cattccatta ttttttagct gtttattcta 300
 aaaaacaaaa attttcagcc actcccatat attctcccat gacatgggtc tatatagcag 360
 tacttaacac agtgccctgc acatagtagg cattcagtaa atacttacat gcatgaatga 420
 ataattgtatt ttcagtgtaa caaatttatt ataaaagtgt agttcgactc ttcttggctc 480
 tggagtttga gagtacagaa ttacagggaa tgaagagagg tataagtaga tattttaatg 540
 gaaatgaagt ataaattaat aacttgactt acccctccat aagttactgc taactggaga 600
 tacctttgta tgcccaacct gtaaaggaaa aagntctatt tctatattta taggacattc 660
 ttctca 666

<210> 84
 <211> 199
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(199)
 <223> n = A,T,C or G

<400> 84
 aanntactta agttattcaa agaatgttat ctttcttgca agagtaattt aagcacatgg 60
 gaaagattct agactttttg tttcttgcaa canacagtgc cctctgctgc tagaaacctt 120
 tttctactta ctatcatttt tattgtggct tgagctcanc tcaatctggt gcagatgacg 180
 ctggacaact actaaccaa 199

<210> 85
 <211> 670
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(670)
 <223> n = A,T,C or G

<400> 85
 cacttcagga gagaacttca tagcacaatg tctttctata agatattttt aatgatttag 60
 tattttacaa catttggtta ccataatttg atataccatt tttttctatc tgcccagttt 120
 tattaataaaa actatatatt attttctaaa gaaacaatca tatttttata caaaattatg 180
 ttttcaggta acgaaataga tgtagggtag agtggaacat aagcagtgtt acccctggct 240
 gggagtcagt attatacaac aaatgggtgag ctggaacatg ccctgtctgt gctgtccctc 300
 ctgtgctggg tcgcggtatg gtaggcaaca ttgccttata acgctagggt cacctgacac 360
 tttaaaagga aaaaaagtgc catagagttc tgtggtcaca aaattgtttt gcttttatca 420
 aatactttta tagaaccaaa gttgcagata ttggaatgta tggaagtatc tcagtctctg 480
 cataagagga ttaaagtatg aaaggatcat ttaatgactg ttttacttat aagtcattaa 540
 gtaatccacc atttcttatg gatgatgctt aagcctgggt aggtttgtac tctaaggagc 600
 ccagatcata atgcagngca tttccttanc ccttagagtt tcttgcaaac atttaaaaaa 660
 agacntattt 670

<210> 86
 <211> 401
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(401)
 <223> n = A,T,C or G

<400> 86
 atcttccac gagtgggatt ctggccttca gagaccagga gggagtgtct gggccgcang 60
 tgtggcactg tggtagaggt gtgtgtcttt gcacacacag tgcagcggga acggtggggc 120
 tggctggtgc tgaagacaga cacactcctg agccaagggt ttgtcttcaa cctccccgtc 180
 ccgttgtecc attttgtctt gtgaagggtc aaatcccttt cttcccttcc catctcaggc 240

```

tctcctgttt tccctcaggg tccagtatgc ctttgagctt tagctgttaa aaaggaaccc 300
ccgtgacttg acacagcttt cacancctggc tgctangacc ggcgggctgg gtgttcacgt 360
gtgtctgtgt catggatgca atgcangccc tgnangactg t 401

```

```

<210> 87
<211> 373
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(373)
<223> n = A,T,C or G

```

```

<400> 87
cttttttttt ctttttttta ttgaaaact actttancaa taattaattc catgattatc 60
acattctgcc attaagggat attagtaccg taatactgaa gaaattttat taagtctgaa 120
cttctggggt aggcagcttc tttgtttctt ttctatccac ccttgctcggg tgaggtatct 180
gtttcttgac taataaacc tttgatactt taaccagaaa tcagctcata aagctatctt 240
tgagtatatg tngggnaaaa aaaaaagggt aacttgggna ataccttcca nnctgacctc 300
cntntaccaa gatatttttc agggctttta tttactatgc nctaanacta tgcncctttt 360
ctgaaatatt ttn 373

```

```

<210> 88
<211> 507
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(507)
<223> n = A,T,C or G

```

```

<400> 88
ccaggagcag cccattcctg atgctttctc agagactcct gcaggcagcc aggccacang 60
acccttggtg tcccacccca cacacgccag attctttcct gaggtctggc tcccttccca 120
cctctctcac tccttgaaaa cactgttctc tgccctccaa gacctctcc ttcacctttg 180
tccccaccgc agacaggacc agggatttcc atgatgtttt ccatgagtc cctgtttgtt 240
tctgaaaggg acgctaccgc ggaagggggc tgggacatgg gaaaggggaa gttgtaggca 300
taaagtcagg ggttcccttt tttggctgct gaaggctcga gcatgcctgg atggggctgc 360
accggctggc ctggcccttc aggggtccctg gtggcagctc acctctccct tggattgtcc 420
ccgacccttg cctctacct gaggggcctc ttatgggctg ggttctacct aggtgctagg 480
aacactcctt cacagatggg tgcttgg 507

```

```

<210> 89
<211> 796
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(796)
<223> n = A,T,C or G

```

```

<400> 89

```

```

gactttaatg ggnatatggg ctctacactc atagggaccc acatgttcca agcctccagc      60
tgtgtgttct gcttcctcaa gcagcagctg actctgatat tccccatctc accttagcag      120
tctatatcaa gcaaagggca tgcagaatcc catgcaccca tcatgtatgt aataaaaggc      180
agatattatg tggctctctg aaccagtctt aggcattggag gttgaggatc aggagtgact      240
tgagggtact gactggcaga gcaggagccc cgttattttg gacaaacacc gccactttaa      300
gtttcagctt catttttagc cttctggatt taaggaaatt acttttttaa aaactataag      360
cagccaaaaa aagcagacag taaaatgcag ataaaacagc tcgggcacag aggaagggtg      420
aggaaaaagtc tcttgggtaa ctgccaaact tcacctcat acaatgggcc ccagtaaaac      480
agtgggcctt aataagcaca ttcccttccc tccaggtgca ctaaaatagg gaagctaaaa      540
gcagacttgg ggggtatgcc tacagctgca gaaaaatgta taaaagcaaa cacaccactc      600
tccctcccat ataagcacia caaaaaaaca cagaagcagt ccaagcctnt aagaaactct      660
cccaccctaa atccttaaac actcttagtc tgtagaaaag actctaacct aattcagcca      720
gcagcccttc tcaggtgtgt tttntntaaa ataaacctgt nttaaccatc aagccaaaaa      780
aaaaaaaaaa aaaaaa                                     796

```

<210> 90

<211> 462

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(462)

<223> n = A,T,C or G

<400> 90

```

tagaacatnc tgaatTTTTT ttgtactgnt ggactntatt cagtgtcatg tectatatct      60
gatcaagttt tcaagnagat aatTTTanaa tgaaaaagaa aatcctcttg tnggaaacaa      120
aagacgtttt atatgtgcag tatgacaaan aggagtttca nagacaactt tgaatccttg      180
tcagcctgga gaccagcnc agaggaatnn ccaaggcaaa ctcccatata ttgtcttccc      240
ccaaattgct gccctacag actcaaaagct ctttttcttt gttttgttgt ttntctaaaa      300
atttactgtt ntttgtcgat gctatataag ccaggggagt ttaagacgcc agctntttga      360
natttntca ttcccttgta ttcccatcat anatattaca tatacccgng taataaattt      420
atgtttgtta aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aa                                     462

```

<210> 91

<211> 591

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(591)

<223> n = A,T,C or G

<400> 91

```

cgggccttgg aagcctttga cctggatcct gcacagtggg gagtcaatgt ccagccctac      60
tccgggtccc cagccaacct ggccgtctac acagcccttc tgcaacctca cgaccggatc      120
atggggctgg acctgcccga tgggggccat ctccccacg gctacatgtc tgactgcaag      180
cggatatcag ccacgtccat cttcttcgag tctatgccct ataagctcaa ccccaaaact      240
ggcctcattg actacaacca gctggcactg actgctcgac ttttccggcc acggctcate      300
atagctggca ccagcgcta tgctcgctc attgactacg cccgcatgag agagggtgtg      360
gatgaagtca aagcacacct gctggcagac atggcccaca tcagtggcct ggtggctgcc      420
aagggtgatt cctcgctttt caagcacgca gacatcgtca ccaccactac tcacaagact      480
cttcgagggg ccagggtcagg gctcatcttc taccggaaa gggngaaggc tgtggacccc      540

```

aagactggcc gggagatccc ttacacattt gaggaccgaa tcaactttgc c 591

<210> 92
 <211> 647
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(647)
 <223> n = A,T,C or G

<400> 92
 gaataactag aaattttattg gatcaggttt cacatttgca tttttgaaaa ctactaccaa 60
 aaagatttca ccaattttaca actccatcat tagtaagaat gcctgtttgc ctatagtctg 120
 ccaaccctga atccttaaaa atttttgcca atctggtagg caaaatttct ttcttttctt 180
 tgaatattaa tgaggaggaa catcttttca tgtttcttgg ccatttgcac ttcttattat 240
 gaattgcttt tgcccatttt ccttttttta attatgaaag tctaatgact accttctcat 300
 tgtataaaaa acacagtctt ttgaatagag agaccctttt ctccaatgct accaatcaca 360
 ttccacttac cacagtttta catacatcct ctatgcacct ttccgtacga atatacatat 420
 acataaaaaac acttttttaca taaataggat ctcatattct gtagcttttt aaaatttttg 480
 nctcaaaaaa aganaacang gctttaaatt tctttaatgg gtgaatatga ataaatacta 540
 tgaaaatgcc attatttatt cccttaattt ttttcctctc gctattacat tgccaaagna 600
 aacatcctat tcanatgtct ttgnngcatgt gngngaatat ttctttta 647

<210> 93
 <211> 740
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(740)
 <223> n = A,T,C or G

<400> 93
 gtcgatag aggaaattaa tgcaaggaaa gaaaacaagc ccagttgggtt tggcttgtgc 60
 tactgcaagg ctttacaatc agattatata acatacatag atgaactcct gacctatata 120
 aatgcaaaac ccaacctgtt ctctatgctc ctaacggatc cacatctggc tctgaccgtc 180
 ttctttggcc catgctcacc ataccagttc cgcttgactg gccagggaaa atgggaagga 240
 gccagaaatg ccacatgac ccagtgaggac cgaacattca aggtcatcaa agctcgagtt 300
 gtacaagagt ctccatctcc ctttgaaagt tttcttaaag tctttagctt tctggctttg 360
 cttgtggcta tttttctgat ttctctataa gtaaaagatc tcctaaatgg aagatgcaca 420
 gagtagattt acaatgctcc aattcctctc ttacagcaat attgccttca cagttataaa 480
 ctgtattcaa atagtaaagg ccaccctctc gcttccttgg ctggccccag ggctaccact 540
 ggtattcctg agcctctccc agctccactt ctaatgctag agaagataa ctaagacttc 600
 tgtgcatttg aaggttggtg gaaagttaca gggttcattt anaaagaaaag ctgttcttga 660
 cagcactctt gagccatcat acctctttcc ntataaaacta ttttccagaa ctcnactaaa 720
 accccttact tcacaaatga 740

<210> 94
 <211> 608
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(608)
 <223> n = A,T,C or G

<400> 94
 ttttacaatc cttaggaaggc ccaccaatatt cattttcacgc gccagggcggt ctgcagttgg 60
 aggccgaggg cagccctctg ctcactgaat gtcttgcatg tgctgactgc tgcccgcagt 120
 gctgaacatg cccacccgcc caggcccagc actgcttggt gggtcagcat ctagtgtctgc 180
 tgtcacatct ttgtctgcac agccagtagg attgcctcag ccaggggggt tatcagaagg 240
 tgtgcaaggc ctttggggga actgagcccc tatagtgggc agtctccttt accttcccac 300
 ctccctgaaa agcacagaag acagtgcctt ggtttgtgtt ttgaagcaaa caagtcagct 360
 ttctggcttt gccccaaaac tgtgatggaa cataataaaa ctggagatat ggtttttaac 420
 actgcaaaaa ggaaaaagca tcaagtttct acttctggct ggaaagcaaa accaatctca 480
 gctgacaagg ctgggcaaac taagttttcc tgagcccatt ttcctttgag ccctgacctc 540
 ncctgcctta cctcattaag gtttgggtta agcantggaa aggagganga ngcanggggtg 600
 gatggggg 608

<210> 95
 <211> 706
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(706)
 <223> n = A,T,C or G

<400> 95
 agaataatga gtacacaagt ttttgccat ccccttcctt aaaatataag aaataaagga 60
 gctcttacga aaaacccagg ttagaccgca taaaaataa agtgaacagt gaggtggtag 120
 caagacttct ttttagaaaa gaaagcattt acctgcctgt ctgtaagggt gaaatttcat 180
 cagtttgcaa acgataagaa atgcagactt gctcttgata gaaatgctta gaaacactct 240
 ggggggggaa aaagctcctt ccataatactg tgagacattt gttaagtga atctattgtt 300
 tatcagcttt taaggataaa aaaggtattt taaaagttgg atatttagga tatttgagga 360
 tattccttta tgagctctcc atatccttct tgagaaactg gttaaaaaag gaataggggt 420
 tgagtgttac agagagttag ctgaagattc ctgtgtaaaa gcaaagctaa caagcaatga 480
 agacatgaag caaaatacta atctaattgt gtaaaagaa gatattttta taagttcttt 540
 ctgcttgctg ctaagagttt gctaaagtgt catgaattat tctggttatt actaaagttt 600
 ctatgaaaca agtagatttt aagaataaat gtttctggaa aagaactatg ttatgatttt 660
 gtanaaatgt aaagattact tgaggtgttt aaaataaatt tttcat 706

<210> 96
 <211> 719
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(719)
 <223> n = A,T,C or G

<400> 96
 ccagggcctg tccctgcccc agtggcggtt gcgctgggtt caggcccagg gggccctgna 60
 gaagctgtgc agctgaagag agggttcaaa cggaagccga gaacttgaca ctgttcaccc 120


```

caacacctca cctccccag gacatttga agaaagcagc gccaggatc ctcggcagtc 180
gtccccaccc gcacctgcag tcccctcatg tgctgttctg ctgcccact cagctcctgg 240
acctgtcct ttcacccgc taaagcacc cctaaaacce cttcatcact ttcattctca 300
gcaaaaagta attgagcac tctctaggg gctggggagt ccacactgaa caaaagaaac 360
agaaaaccct gtcttcagc agttgagtt tagggcaggg agacagagtt tacaagataa 420
ggaaaatata tatgtagtat gctgcaagtt aactgctgtg tgggaaatcc agcaggggggt 480
gggatgtgtg atttgaattg agggccacac tgcccaggtc gtgctccgtc aaggggtgag 540
caggagcaac aggggtggct gagtaanggc ttgcagctgg aggcaacagc acatgcaaag 600
gccctgagcc aggatgtgct gcaataangg ccactgaggg ggacagtgtg ngttgggggg 660
tgangaatgt attaaaaaga ngaaaaattg ccttttaaaa aaaaaaaaaa aaaaaaaaaa 719

```

```

<210> 97
<211> 572
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(572)
<223> n = A,T,C or G

```

```

<400> 97
gcttggtctaa tttccaaatt attgcataat atgttctacc ttaagaaaac aggtttatgt 60
aacaaagtaa tgggtgtgaa tggatgatgt cagttcatgg gccttttagca tagttttaag 120
catccttttt tttttttttt ttttgaaagn gtgttagcat cttgttactc aaaggataag 180
acagacaata atacttcact gaatattaat aatctttact agtttacctc ctctgctctt 240
tgccaccgca taactggata tcttttcctt caaaggaccc taaactgatt gaaatttaag 300
atatgtatca aaaacattat ttcatttaat gcacatctgt tttgctgttt ttgagcagtg 360
tgagtttag ggttcatgat aaatcattga accacatgtg taacaactga atgccaaatc 420
ttaaactcat tanaaaaata acaaattagg ttttgacacc cattcttaat tggaataatg 480
gatcaaaaat agnggntcat gacctacca aacacccttg ctactaataa aatcaaataa 540
cccttagaag ggtatgtatt tttagttagg gg 572

```

```

<210> 98
<211> 520
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(520)
<223> n = A,T,C or G

```

```

<400> 98
tcagcctggg gtcctccact tctggaggcc tctaggetct atcagcaact gggggacana 60
acagcagagc tggagagtct ggagctgcta gttgaggcct tgaatgtccc atgcagttcc 120
aaagccccgc agtttctcat tgaggtagaa ttactactgc caccacctga cctagcctca 180
ccccttcatt gtggcactca gagccagacc aagcacatac tagcaagcag ggcaggagac 240
gctgcagagc attacttgga cctgctggcc ctggtgctgg atagctcgga gccaaaggttc 300
tccccacccc cctccctccc agggccctgt atgcctgagg tgtttttgga ggcagcggta 360
gactgatcc aggcaggcag agcccaagat gccttgactc tatgtgagga gttgctcanc 420
cgcacatcat ctctgctacc caagatgtcc cggctgtggn aagatgccan aaaangaacc 480
aangaactgc catactgncc actctgggtc tctgccaccc 520

```

```

<210> 99

```

<211> 470
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(470)
 <223> n = A,T,C or G

<400> 99
 tttttttcaa agcaattgtg acacctacct gtggaccaag gaaaaaacca aatcatccnc 60
 aatctttcag ttccacaaca acaccattat aaaaacgagt tgatctgaag tggttccaaa 120
 tctttcttat cttcaagatc tatgattagt aattctgact cgttgcaaag caacatattt 180
 tttgagatac tgttgtgagc cccgggagaa tgtcagagtc ctgtccactt ggacatgggt 240
 ggaggggagg ttgggcaggg ctgataaaaag actagtacgc ctctgtgtctc ttcagtctta 300
 ttttgcaaac atcatctata aagggtttttg ctactccaag ttttggttagc ccaagctcat 360
 caaagcatgt gtctattatg tgnctancat agtaaaaatg gctctaaatt gcatataaat 420
 gccagattt taataatcta ttgnttcana agaaaacaac agnannngt 470

<210> 100
 <211> 570
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(570)
 <223> n = A,T,C or G

<400> 100
 agtttttatt tttttctgta acgcccaccc tgcttgcagt agaccctttg ggcttaagan 60
 gtcctacaga ttctaatagt ttctcttttg tagatgtgtt aacatattta aaatacttag 120
 gacatggcct ggtacgtgac agatggtgtt taacatcatt gtcatatgaa gaacactctc 180
 tgtgggcaa tggaggtgtg ccttaaaatt cttcatcttg cctgattttg tttgcataac 240
 ttctggagag tctgtgtcct cctcatctag gccacctttc cattttttgt gaaagatgac 300
 cttgtccctg cagtctccgc caggagtcac acagcttttc acagcagctg ccactcttaa 360
 ttctttttcc tgagattcca gcaagggtgt gacattgtca cttttttgtt ctagactctt 420
 ttaaattttc tgcatttgcc tgaaaagcac ccctgtaaga atagatttct catggctcta 480
 aaaattattc ccaagaatac cttacttggg tcaaaagcag actgtttctc ttcatttcat 540
 ctcaaatcan acttctgggc aagatgttct 570

<210> 101
 <211> 365
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(365)
 <223> n = A,T,C or G

<400> 101
 atcaataaac caaggtaatg cctnagggtt ccctcccagt cctcncatca gctctggcct 60
 catcaccaag gtcacanagn acacagggga gggggaanac ccacncacac tccttggaat 120
 ggtcctgtt atttatgctt ggngcncagn catattanaa gaaaaaaaaa agctttgtat 180

```

tntttttccn catatnatgg ntgctgttta cacaccctgc caatgcntta gcnntggaga 240
gcttttttgca atatgnnggg gaaaggggag ggaggggaatg aaagtgccaa agaaaaacatg 300
tttttaanaa ctnggggtttt atacaataga atgttttcta gcagaaaaaa aaaaaaaaaa 360
aaaaa 365

```

```

<210> 102
<211> 546
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(546)
<223> n = A,T,C or G

```

```

<400> 102
antgacacac tagcccccctg ccgcctcggc atgaacagca tgggcaccat ggatggcacg 60
ctgatgggtgg taggaagcan aagggctcct gggcagaaag ggcttggtnt gaggtgaaac 120
ccnccttca agcctgggat ggcctgaggc ctgggggccc ggctgccagc tntgggtagt 180
ntgtgggtgg agtganaatt tatggtgctt tttccgggcc tgctcatgga ccaatcagca 240
tgcacttctt cccttntgag ccataaaaa ccctggattc agccagactt ggacanatgt 300
caatactacc aactgtggga agaagctacc cacttcagga ctcttgact cctcaggatg 360
acctgcctac anaaagaage taccactat gggtnntntn tntgctgana gctggatact 420
ngtcaggatg acctgcctgc anaaanagct accnccatg ggtntcctnt gaactgttct 480
gttgcccaat aaagctcctn tcctttttgc tcnccctcca aaaaaaaaaa aaaaaaaaaa 540
aaaaa 546

```

```

<210> 103
<211> 376
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(376)
<223> n = A,T,C or G

```

```

<400> 103
ctcaactccc ncttcacctt atgtaatgtc tgggcctgag atttctctct ggctttantt 60
tcttgatgtt cctgtgatat ggcttctgcc agcaatgaaa acaaggctct tatagtagtn 120
ctccatggag tccagttcat cctggtggcg tctcctttgt tcctctcget tttctttggc 180
atagtttctt aggtctcgta atctttgctt ttgaatgttt aaaccttctt caaacagtgtt 240
cttaaatatc atttcttccc gggtcctcat tctcatcatt ttgacacaca actgaactct 300
ataatcatca taatattttc gagcacgaac gatttgctgn cgatnttctt ttatctttga 360
ttgggncaac ctttgc 376

```

```

<210> 104
<211> 700
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(700)
<223> n = A,T,C or G

```

```

<400> 104
tttgaaaata cagatTTTTT ccaacttttg attctTTTTT agttatatgt ttgtctttcc 60
tttttaaatt gttcaaaact attttttaat ggtcaagtta ctaacacttg aaaatcagat 120
actgcaccaa atacagtgtt tttccgtagt gtttttaatg agtgcaccta ttactactgt 180
gcgagaattc atgttttacc agtcattgtt atattacaaa cagacttgca tgattaacca 240
gttgttacac ttactttttc aagttggagt atatatgact cagtgcagac tggctctctc 300
tatgtgaatg cacacatgca gaaatgcaga gtcaatttta catgccata aagacatttg 360
taaagaattc agctcttatg gtctgttgta taaatgtgta tctaggcact ttggaatttg 420
acctcacaga tgttacaact tgatcagtcg tttgacctaa tttgtggnag ctatctgtat 480
gttttgcaat ctttaatacag acatgctttc caaaaagatt aatacagaac catcctgccg 540
ttttggataa gtctatccag ctgtggaaaag ggcaacctgt ggtttctctg tactgggtgt 600
taatggggga agaatatgaa cagctttaaa gagctgtgta ttgggggtac tactattaaa 660
aaataagatc tgcacgagtc tgactggcct ttgggtggcc 700

```

<210> 105

<211> 729

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(729)

<223> n = A,T,C or G

```

<400> 105
taaaaccttc acactgagaa ctgcttgtgt gggagagagc tggttgggtt gatcttttcc 60
gagtgtgact tacctccttc aaggggatgt ttaagcttct cgggcagaag tgggtgtgtc 120
attcctgaca ccaaaccacc tggtatatgt ggttgtcaca ctcagctagt gatgataaag 180
gtgttcttaa atatgttagc tttcagtttt cctgaggaag caattttatg gatacttccc 240
cctccttctc aagtgaggaa tagcagagca aattttatgt ggaacttaaa ccaatagtta 300
taaccaatag tttcaacctc ctgcctcacc actgcttctc tctgagctc tttccccaca 360
cctcaaaaag agtacaaaag gattccatct gcagaggtaa attctttgtt taaaaaagta 420
ctgtttttct tatcttttct ggttctccta ggtatcagaa caaggtttaa tagaaatcct 480
taaaaaagta agccaacaaa cagaaaagac aacaacagtg aaagtaagtg tccccanatg 540
cttgtggcaa atgaaaagat ggatacttta aagattaatg ttgagtatac atctaccaca 600
catatttttc agcccanaga cattttcctt ttgtcaaaca cgtgaaagtt tggggagaaa 660
ngctgaatct gttgggggag ggttctaatt tttatagget cttgactcca ttccccctt 720
ttaattcac 729

```

<210> 106

<211> 481

<212> DNA

<213> Homo sapien

```

<400> 106
gaaaagcaga gtcagctact gagagctctg gaaagaagga tgtcaagaag gtgaaatcct 60
aaagcctaga aataaagttt taaatgggaa actgctatct tcttgttccc atcttcaaatt 120
gctaattgcc agttccagtg tattcatggt actctaagaa aaatctcttt ggttttgatt 180
tcttgcatat tttatatatt ttacaatgct ttctacctga aatgtgtagc tttatatatt 240
atggcattct agtatttttg tgtactgtat tttgtgcatt tcatgtcttc atcaaaatcc 300
tctcagtcct tgttcttttg aagcttgtgc tgagggttta gcttttctat gtttttatgt 360
ccgtgctttt gaaagagAAC ctgatttcta tagttgtatt attgttgttt catactttaa 420
atztatatgg ctgtggaaaa acgaattaaa atgttttgag gagaaaaaaa aaaaaaaaaa 480
a 481

```

<210> 107
 <211> 519
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(519)
 <223> n = A,T,C or G

<400> 107
 cagctcctct cctgccagag ctaggcaggc gccgaagtag ccgcatggcc ccgtcagant 60
 accccaggga ctggagagcc aacctcaaag gcaccatccg tgagacaggc ctggagacca 120
 gctccggtgg gaagctggct ggccatcaga agaccgtccc cacggctcac ctgacttttg 180
 ttattgactg caccacaggg aagcagctct ccctggcagc aaccgcatca ccacccaag 240
 cccccagtc caatcgaggg cttgtcacc caccaatgaa gacctacatc gtgttctgtg 300
 gggaaaactg gccccatctg actcgggtga cccccatggg tgggggatgc cttgccagg 360
 ccagggccac cctgcgctc tgcagagggt ctgtggcctc agcttccttc ccagtcagcc 420
 cgctctgccc ccaggagggt cccgaggcta aggggaaacc cgtgaaggct gcgcctgtga 480
 ggtcttcaac ttggggaaca gtcaaggact cactgaaag 519

<210> 108
 <211> 669
 <212> DNA
 <213> Homo sapien

<400> 108
 ggacaatgaa gactgaacta tcgcacatta cctaagaaag atgggaattg acatgcacat 60
 cacaattgta tacacaacag aaattattga atcatgagat atacattccg gtgtgtgaca 120
 gattggcaca tgacataatc tgggttcttt atagactcag ttgttttggg gcgatctaga 180
 ttatcaagag aagagccttc atgcttagct ttatcttgta ccaaccacca gggcccttgc 240
 ttctgagcag gaagcagctg gggaataggc tctttctctt aatgacttcc aacatagtgc 300
 tctcaaacct tactcctcca gaaggccacc ctcacctggc tatggctact tcagaaaaaa 360
 cttggcctct ggtataatag agcagaatca tcacctcaca ttctatttca agccaaagtc 420
 aatatctcaa aggcctgggtc tgtgatttat ttggctcttg ggagctccta ctgaaagtgc 480
 tgaaatgtcg tactgacact tcagacttat agctacctag actccaagta agatttatct 540
 ctgactggag ggtttctcct attaaaaacc aaagagtgtg ggggtgcctc acctgctagg 600
 taatcttcta tgcctaatg ggaagaatgg gagcagcaga caagtaagtg caggaaggag 660
 aaccaaagc 669

<210> 109
 <211> 349
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(349)
 <223> n = A,T,C or G

<400> 109
 tttaagccg gatatttgggc ctgcttaaacc cacttaaagt tanttaatga cagatggttt 60
 gaggtttaaa agtcttctgg agaaagcccc ccagagaaca ttccctttga agccccatgt 120
 aaaaatacgt gtgggagaga aagtgttttc tctgacttct gctgacagtg gctaaaactc 180

tgaactgtca	ggagtattca	aaataagact	gcctttagg	taagcctgtg	gtagcttttt	240
tgagcacagg	ataaaatact	tgagtctttg	cttaaagtgt	actttctcaa	tgaggctttg	300
tatgactaaa	taaaatctgt	ataatcccca	aaaaaaaaa	aaaaaaaaa		349

<210> 110
 <211> 337
 <212> DNA
 <213> Homo sapien

<400> 110						
ggcctttccc	actgggtccat	ctgggttttct	ctccagggtc	ttgcaaaatt	cctgacgaga	60
taagcagtta	tgtgacctca	cgtgcaaagc	caccaacagc	cactcagaaa	agacgcacca	120
gcccagaagt	gcagaactgc	agtcactgca	cgttttctac	tctagggacc	agaaccaaac	180
ccacccttcc	tacttccaag	acttattttc	acatgtgggg	aggtaaatct	aggaatgact	240
cgtttaaggc	ctattttcat	gatttctttg	tagcatattg	tgcttgacgt	attattgtcc	300
tttgattcca	aataatatgt	ttccttccct	caaaaaa			337

<210> 111
 <211> 552
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(552)
 <223> n = A,T,C or G

<400> 111						
atattgtcaa	gtttctttaa	tggtgaaca	gaaagaagct	tcaagtaatg	gagaaggcat	60
tgtctgagtg	cagctgcttt	cctggacgcc	tgtgccgttc	ctgtcttcca	aatcctatgc	120
ttggaggccc	ctggaggtac	atatttgcca	ggaaccaacc	tgaccttaaa	aagatgagtg	180
tgacacagcc	ggctgggcag	gaggatggag	gtgccacagg	acaccacctg	cccacgccct	240
ggccagcctg	gccatgctgc	cgagtgcggg	gaggccaccc	caccagagg	gcacagggca	300
aaccctaagc	acgggggtat	tgcccttgaa	gccccagggg	atgccctgtg	ccggatcctc	360
atgcctcatt	gactagcctg	cttgctgaag	gagcccaggg	gcctgagcct	gcaacactgc	420
aaggggtgag	aagggcacgc	tgctgtgggc	gccactggac	tcaaacctca	cattanaagc	480
tacaaagaac	cccaaagtcg	cttcaanagc	cccaccaacc	cccaagccan	gtcatncct	540
gacacantga	at					552

<210> 112
 <211> 115
 <212> DNA
 <213> Homo sapien

<220>
 <221> misc_feature
 <222> (1)...(115)
 <223> n = A,T,C or G

<400> 112						
gtcctaggtg	canaaacttc	ccaaacaana	actatgcncg	ancnctaccc	cttcgagtg	60
tctnctttct	gtcctgggtc	ccatggnttg	ccccatttta	taaggtagcg	tgga	115

<210> 113
 <211> 649

<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(649)
<223> n = A,T,C or G

```

<400> 113
gcctgattaa aaactaagca gaagtagttt taacanaaat actcatgaaa atgtttngaa      60
actgaaatgt aaacaactgt aatattaagg aaaccagaat caataaatca ctgtcttgcc      120
agcacagcta cagagtaaca tgattcaggg gaggaaggt ccttagagtt acttttataa      180
ttcttttttt ttctctctta ggtttanaaa tcttacaaat ttaaacttta tccttttaaa      240
attatttgaa cataatttag atattgtaag cttaaaatac aaatgtttat agataacctc      300
tttaccataa actaatccct ggcaagccat ggctctcttt ttttttggg gttaaagcc      360
tgtaaacagt tttctgaat gatcatgaac tttcttggg tttagcacta ggatttagct      420
atgaagagag ctcataggct ttcaggtgct aattgagatc tgccctgtta gagtctggg      480
gtgctagatt ggtcacattg acaccagtgg cagggaggcc atctatgagt ttgatgcttt      540
ttatcacaca cttcagntgt ttagaaagtt attaccaata cttttaaaca acactccaag      600
aaaatttgct atatttcttt ctcatcacta cagagagagt agatttccc      649

```

<210> 114
<211> 650
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(650)
<223> n = A,T,C or G

```

<400> 114
tggcgattgg tggtggcggg ctggctcagc tgggcagggg gtaactttac tgatttgggg      60
gtggttttta gtttaatttt tcttttctag ctccccatcg acggtcagtg cgcacgttgt      120
aatcagctga ggccatgtca ggagacggag ccacggagca ggcagctgag tatgtcccag      180
agaagggtgaa gaaagcggaa aagaaattag aagagaatcc atatgacctt gatgcttgga      240
gcattctcat tcgagaggca cagggttagt gatataggat tacatttcct tctctatggg      300
tccaatcaca ctacttggtt ctgcagtga taatattttc ataatcctaa cattgtaaat      360
gctgtttatt ggttttcaat tttagaatca acctatagac aaagcacgga agacttatga      420
acgccttggt gcccgattcc ccagttctgg cagattctgg aaactgtaca ttgaagcaga      480
ggttactatt ttattttatt ttttcttata tcagtatttg cagcattcac tgtagtgata      540
gaaaacaaag ttangaacat agccaattan gacaaggagg atttaaagt gtettacctt      600
tattttgtaa aataggtata aaggagtaat taaaatgaat ttttgaaatt      650

```

<210> 115
<211> 403
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(403)
<223> n = A,T,C or G

<400> 115

```

gtattatgac tttaaaaacc ccattattga aaagtacctg acaaggcagc tcacgaancc      60
caggcctgtg atcctggacc cggcggaccc tacnngaaac ttgggtggng gagacccaan      120
gggnnggagg canctggcac angaggetga ggcctgnctg aattacccat gctttaagaa      180
tngggatggg nccccantga gctcctgnat tctgctgggtg anacctcctg cttcctccct      240
gccattoatc cctgccccctc tccatgaagc ttganacata tanctggaga ccattctttc      300
caanaaactt acctnttgcc aaaggccatt tatattcata tagtgacang ctgtgctcca      360
tattttacag tcattttggn cacaatcgag ggttnctgga att                        403

```

```

<210> 116
<211> 397
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(397)
<223> n = A,T,C or G

```

```

<400> 116
cgaaagaaaa aaagtgatat tggaccctgg aaagattttg aaacttgagt ggtttgataa      60
cccttctatg tattgtaggg agaaaaaaaa aagtttattt tattccactg tcctccctta      120
aaagcatcat ttgancaata aatgaatatt gtctttaaac caagggttag ggaattttcc      180
tctctctctc tctctcctct ctctttctgt tcaaagaact tcaaacattt gggaccacct      240
ggtattctgt attttccactg gccatatttg aagcagttct agttgcattg tattgagttg      300
tgctggcagt agtttccatg cctgtcaatg tatcatagtc ctttgttgcc cagataaata      360
aatatttgat acgcttttaa aaaaaaaaaa aaaaaaa                        397

```

```

<210> 117
<211> 59
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(59)
<223> n = A,T,C or G

```

```

<400> 117
cacttatggg gacaatggga agactcttct tttncaactgg actgtacctg gacntnnaa      59

```

```

<210> 118
<211> 751
<212> DNA
<213> Homo sapien

```

```

<220>
<221> misc_feature
<222> (1)...(751)
<223> n = A,T,C or G

```

```

<400> 118
catcaacata cccgctttat tgctgactca tgacaactaa tgggaagaca tggctcagat      60
gtgcagccac agtgagcttc tgaacatttc ttctcagact aagctcttac acacagttgc      120
agttgaaaga aagaattgct tgacatggcc acaggagcag gcagcttcct gcagacatga      180
cagtcaacgc aaactcatgt cactgtgggc agacacatgt ttgcaaagag actcagagcc      240

```


aaacaagcac	actcaatgtg	ctttgccc	aaattacccat	taggtaaate	ttccctcctc	300
ccaagaagaa	agtggagaga	gcatgagtc	tcacatggaa	acttgaagtc	agggaaatga	360
aggctcacca	attatttgtg	catgggttta	agttttcctt	gaaattaagt	tcaggtttgt	420
ctttgtgtgt	accaattaat	gacaagaggt	tagatagaag	tatgctagat	ggcaaagaga	480
aatatgtttt	gtgtcttcaa	ttttgctaaa	aataaccag	aacatggata	attcatttat	540
taattgattt	tggtaaagcca	agtcctattt	ggagaaaatt	aatagttttt	ctaaaaaaga	600
atthttctaa	tatcacctgg	cttgataaca	ttttctcctt	tcgagttcct	ttttctggag	660
tttaacaaac	ttgttcttta	caaatagatt	atattgacta	cctctcactg	atgntatgat	720
attaagttct	attgcttact	ttggatttct	a			751

<210> 119

<211> 591

<212> DNA

<213> Homo sapien

<400> 119

aggcttcttg	tcacactgaa	cacatccagc	cacaggcacc	agctggttgg	gaccagcagc	60
ccccagcatc	ctcttgca	ggctggcaca	aaaagaaacc	tgctgtatac	ccccaaagt	120
gtccctttcc	caattacctc	tgggtctctc	tgctgcttgc	ctctgctgct	ctggactggg	180
agagcttctg	tcctgtgtcg	catgggtatt	tagactgtgg	gggagatgcc	ccttcttata	240
gcactggagg	aggaaaacaa	attcttgtcc	ccctcagaat	gagagtggct	ctttctgatt	300
tgcaaggcca	ctatggtcag	ggcaaaggca	tggcccaggt	gtttaagtac	aggtgacgt	360
gtgcctatgc	aatgggggtgg	taaggcaggc	acgaagagtc	caaaaaatct	aggtggcctc	420
tcagctctgc	cacctctagc	tgcatgacct	tgggcaagct	atgtaacccc	aattgcctgc	480
tccattaaag	actgtgaagg	tagaatgttt	gtaaagctct	taacagtatg	taagccttca	540
ataaatttca	gttttccctt	tgttttcttg	atcaaaaaaa	aaaaaaaaaa	a	591

<210> 120

<211> 652

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(652)

<223> n = A,T,C or G

<400> 120

atthttgcagt	acattaaaaac	tgaggcccag	agatgtgatt	tgcttgaggc	cacacagcta	60
gattttttggt	ggaagtgggc	cttgaacaca	gtgtactttc	tgagtttct	gactgtaaaa	120
cccagtgtct	gctctctgag	ttccatttcc	aagccccctt	ccatcttggg	cctatgtggt	180
ctccaccata	ttcacacacc	accgccacca	cttgccaatg	cctctcttaa	agcaatatac	240
ccattcgctt	ctttattggg	aactggatgg	atgaagcccc	aaattcagcc	ccaccacag	300
agaagccttc	ctacactcag	cctctgtcca	cccttggcaa	atctttcaag	ctctctcctc	360
caggaaagtg	gggcccacaac	tcagtcactc	cacccccctt	caggtocctg	aggctggttc	420
tactgtatcc	ccatcacctc	cacaactcca	ctcacccctg	acggctccat	ccacctcacc	480
agttggaagg	cttgtgggtt	cagagaggag	caatgctggg	cagcgtgcc	cagactccag	540
tgthtacaga	tcaccagcat	ttacaaccaa	tccaatggcc	agaagcctcc	tctaaccana	600
aggagttctg	aaggggcaga	tgggggtgtg	agtagtcggg	gagtcgggat	tg	652

<210> 121

<211> 407

<212> DNA

<213> Homo sapien

<400> 121
ctcttttagta tgacactggc aactgacggc actgcggata tgtttggaat aaggttcaaa 60
agaagaggct gttctaagaa agactaccga gtaatcaatc acctcctcag agaaagtctg 120
ctcaggaaac tctctcccca ccagccctcg cacttggttc cttgcgttgc ctctggctgg 180
aagctgttgt ccccaacttc agaaagtgtc tgcagatttc acaggctctc tcttgggtca 240
agggaccagc tctgtgaaca cggcaagtaa caacagagag aaaacgtgag gagaaaagag 300
agaccgggat tttgaaatcc tgtttcttga atgccatctc accaggcaca cgaaagaata 360
aaattaagga atcaagaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaa 407

<210> 122
<211> 752
<212> DNA
<213> Homo sapien

<400> 122
caaatccctc ctatccaggc ttttctgtct ctaatacccc aagcgttacc cctgctcttc 60
cctcattccc ggggctgcag gcgccctcta cagtcgcagc tgtcacacca ctacctgtgg 120
ctgccacagc cccatcccca gctccagtc tcccaggatt cgcctcagca ttcagttcca 180
atctcaactc cgctctgtgt gcacaagccg gtttatcatc tggacttcaa gctgcaggca 240
gtttctgtttt tccaggcctt ttgtccctcc cgggtatccc tgggtttcct cagaatcctt 300
cacaatcatc cttgcaagaa ttacagcata atgcggctgc acagtcagca ttgttacagc 360
aggteccattc agcttcggct ctggaaagct atccagctca gcctgatggg tttcctagtt 420
atccttcagc gccaggaaca ccattttctt tgcaaccaag cctgtcccag agtgggtggc 480
agtgaatact ttttaactttt attctccttc agagcaacat cagaattgcc tgagaactgc 540
aatgaacaat ctgacaaatg tgaagctggc caaaagtcgg aaaatgagaa tgagggtaat 600
cctggagaaa ttgtgacaac aatttgaaaa ttgtggttgc attttaaaagt gtgaacactc 660
ccctatgtaa atatgctgac aataaattgg atggagaatg gtatttaaaa agtggttgga 720
gacttttcac ctgtcctata aaaatttgaa tt 752

<210> 123
<211> 401
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature
<222> (1)...(401)
<223> n = A,T,C or G

<400> 123
atgaaccaa tagaaataag catgcaacat gaacagctgg aagagagttt tcaggaacta 60
gtggaagatt accggcggtg tattgaacga cttgctcaag agtaaagatt atactgctct 120
gtacaggaa cttgcaaatt ttctgtacaa tgtgctgtga aaaatctgat gactttaatt 180
ttaaactctt gtgacatttt gcttatacta aaagtatatc atcttttagt gaatattttc 240
ttttggagag attgtatat ttaaaatact gtttagagtt tatgagcata tattgcattt 300
aaagaaagat aaagcttctg aaatactact gcaattgctt cccttcttaa acagtataat 360
aaatgcttag ttgtgatnaa aaaaaaaaaa aaaaaaaaaa a 401

<210> 124
<211> 103
<212> DNA
<213> Homo sapien

<220>
<221> misc_feature

<222> (1)...(103)

<223> n = A,T,C or G

<400> 124

```
aagataactt gctgggacca catgcctgat tgccacagct gtcacacgt tatttaatga    60
acctagtgtt gaagacagtg aaaaggggtcc attgacngtg gen                      103
```

<210> 125

<211> 1024

<212> DNA

<213> Homo sapien

<220>

<221> misc_feature

<222> (1)...(1024)

<223> n = A,T,C or G

<400> 125

```
gagggcagtg aggagcgagg agcgggcaga ggcagctccg gcggccgaga ggagggagcg    60
cggcgagagagg agggggggct tgcgccccgt agaaatgtca atcagagcct ggacccctgc    120
gtcccgccac cagccccccg ctccgcccgt cagaagccca agatctgggt cctcgcgagg    180
actgccacaa gcccgacaaa cccgcgcgcg tcgctcccg gcgcgggggg gtctccaccg    240
ggggcagcgg tcgcgccttc cgcctgcag ctctctccg ccgcgcgcgc cgcgcgcgt    300
cacagactgg tctcagcgcc gctgggcaag tccccggctt ggaccaaccg gccgtttcca    360
ggccaccgcg ccggcccccg cccgcaccgc ctctccctgc tgggctctgc ccctccgcac    420
ctgtggggac ttcccgagac cgcggggcac ccgggtgccc ccgcgcctt cgtcggcca    480
gcggagcccg aaggcggaac agatcgctgt agtgcccttg aagtggagaa aaagtactc    540
aagacagctt tccagcccg gcccaggcgg cccagaacc atctggacgc cgccttggtc    600
ttatcggtc tctctcatc ctagtctttt aaaaaaaca aaaaaacaaa aaaaactttt    660
tttaatcggt gtaataattg tataaaaaaa atcgctctgt atagttacaa cttgtaagca    720
tgccgtgta taaataccta aaagcaaaac taaacaaaga aagtaagaaa aagaaataaa    780
accagtcctc ctcagccctc cccaagtcgc ttctgtggca cccgcattc gctgtgaggt    840
ttgtttgtcc ggttgatttt ggggggtgga gtttcagtga gaataaacgt gtctgccttt    900
gtgtgtgtgt atatatagc agaaatgtac atatgtgtga accaaattgt acgagaaagt    960
atctattttt ggctaaataa atgagctgcc tgccactttg nctataaaaa aaaaaaaaaa   1020
aaaa                                              1024
```

<210> 126

<211> 214

<212> PRT

<213> Homo sapien

<400> 126

```
Arg Pro Arg Ile Arg His Glu Glu Gly Ser Glu Glu Arg Gly Ala Gly
 1           5           10          15
Arg Gly Ser Ser Gly Gly Arg Glu Glu Gly Ala Arg Arg Arg Glu Glu
 20          25          30
Gly Leu Ala Pro Arg Arg Asn Val Asn Gln Ser Leu Asp Pro Cys Ala
 35          40          45
Pro Ala Pro Ala Pro Ala Ser Ala Leu Gln Lys Pro Lys Ile Trp Ser
 50          55          60
Leu Ala Glu Thr Ala Thr Ser Pro Asp Asn Pro Arg Arg Ser Pro Pro
 65          70          75          80
Gly Ala Gly Gly Ser Pro Pro Gly Ala Ala Val Ala Pro Ser Ala Leu
 85          90          95
```

Gln Leu Ser Pro Ala Ala Ala Ala Ala Ala His Arg Leu Val Ser
 100 105 110
 Ala Pro Leu Gly Lys Phe Pro Ala Trp Thr Asn Arg Pro Phe Pro Gly
 115 120 125
 Pro Pro Pro Gly Pro Arg Pro His Pro Leu Ser Leu Leu Gly Ser Ala
 130 135 140
 Pro Pro His Leu Leu Gly Leu Pro Gly Ala Ala Gly His Pro Ala Ala
 145 150 155 160
 Ala Ala Ala Phe Ala Arg Pro Ala Glu Pro Glu Gly Gly Thr Asp Arg
 165 170 175
 Cys Ser Ala Leu Glu Val Glu Lys Lys Leu Leu Lys Thr Ala Phe Gln
 180 185 190
 Pro Val Pro Arg Arg Pro Gln Asn His Leu Asp Ala Ala Leu Val Leu
 195 200 205
 Ser Ala Leu Ser Ser Ser
 210

<210> 127
 <211> 507
 <212> PRT
 <213> Homo sapien

<400> 127
 Met Ser Phe Pro Gln Leu Gly Tyr Gln Tyr Ile Arg Pro Leu Tyr Pro
 1 5 10 15
 Pro Glu Arg Pro Gly Ala Ala Gly Gly Gly Gly Gly Gly Ser Ser Ala
 20 25 30
 Gly Gly Arg Ser Gly Pro Gly Ala Gly Ala Ser Glu Leu Ala Ala Ser
 35 40 45
 Gly Ser Leu Ser Asn Val Leu Ser Ser Val Tyr Gly Ala Pro Tyr Ala
 50 55 60
 Ala Ala Ala Ala Ala Ala Ala Ala Ala Gln Gly Tyr Gly Ala Phe Leu
 65 70 75 80
 Pro Tyr Ala Thr Glu Leu Pro Ile Phe Pro Gln Leu Gly Ala Gln Tyr
 85 90 95
 Glu Leu Lys Asp Ser Pro Gly Val Gln His Pro Ala Thr Ala Ala Ala
 100 105 110
 Phe Pro His Pro His Pro Ala Phe Tyr Pro Tyr Gly Gln Tyr Gln Phe
 115 120 125
 Gly Asp Pro Ser Arg Pro Lys Asn Ala Thr Arg Glu Ser Thr Ser Thr
 130 135 140
 Leu Lys Ala Trp Leu Asn Glu His Arg Lys Asn Pro Tyr Pro Thr Lys
 145 150 155 160
 Gly Glu Lys Ile Met Leu Ala Ile Ile Thr Lys Met Thr Leu Thr Gln
 165 170 175
 Val Ser Thr Trp Phe Ala Asn Ala Arg Arg Arg Leu Lys Lys Glu Asn
 180 185 190
 Lys Met Thr Trp Ala Pro Arg Ser Arg Thr Asp Glu Glu Gly Asn Ala
 195 200 205
 Tyr Gly Ser Glu Arg Glu Glu Glu Asp Glu Glu Glu Asp Glu Glu Glu
 210 215 220
 Ser Lys Arg Glu Leu Glu Met Glu Glu Glu Glu Leu Ala Gly Arg Gly
 225 230 235 240
 Gly Gly His Gly Gly Arg Gly Ala Gly Arg Arg Arg Arg Asp Glu Glu

Ile	Asp	Leu	Glu	Asn	Leu	Asp	Ser	Ala	Ala	Ala	Gly	Ser	Glu	Leu	Thr
245								250				255			
Leu	Ala	Gly	Ala	Ala	His	Arg	Asn	Gly	Asp	Phe	Gly	Leu	Gly	Pro	Ile
260								265				270			
Ser	Asp	Cys	Lys	Thr	Ser	Asp	Ser	Asp	Asp	Ser	Ser	Glu	Gly	Leu	Glu
275								280				285			
Asp	Arg	Pro	Leu	Ser	Val	Leu	Ser	Leu	Ala	Pro	Pro	Pro	Pro	Pro	Val
290								295				300			
Ala	Arg	Ala	Pro	Ala	Ser	Pro	Pro	Ser	Pro	Pro	Ser	Ser	Leu	Asp	Pro
305								310				315			
Cys	Ala	Pro	Ala	Pro	Ala	Pro	Ser	Ser	Ala	Leu	Gln	Lys	Pro	Lys	Ile
320								325				330			
Trp	Ser	Leu	Ala	Glu	Thr	Ala	Thr	Ser	Pro	Asp	Asn	Pro	Arg	Arg	Ser
335								340				345			
Pro	Pro	Gly	Ala	Gly	Gly	Ser	Pro	Pro	Gly	Ala	Ala	Val	Ala	Pro	Pro
350								355				360			
Thr	Leu	Gln	Leu	Ser	Pro	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	Ala	His
365								370				375			
Arg	Leu	Val	Ser	Ala	Pro	Leu	Gly	Lys	Phe	Pro	Ala	Trp	Thr	Asn	Arg
380								385				390			
Pro	Phe	Pro	Gly	Pro	Pro	Ala	Gly	Pro	Arg	Pro	His	Pro	Leu	Ser	Met
395								400				405			
Leu	Gly	Ser	Ala	Pro	Gln	His	Leu	Leu	Gly	Leu	Pro	Gly	Ala	Ala	Gly
410								415				420			
His	Pro	Ala	Ala	Ala	Ala	Ala	Tyr	Ala	Arg	Pro	Ala	Glu	Pro	Glu	
425								430				435			
Ser	Gly	Thr	Asp	Arg	Cys	Ser	Ala	Leu	Glu	Val	Glu	Lys	Lys	Leu	Leu
440								445				450			
Lys	Thr	Ala	Glu	Gln	Pro	Val	Pro	Arg	Arg	Pro	Gln	Met	Arg	Leu	Asp
455								460				465			
Ala	Ala	Leu	Val	Leu	Ser	Ala	Leu	Ser	Ser	Ser					
470								475				480			
485								490				495			
500								505							